October 2011

miScript™ miRNA PCR Array Handbook

miScript II RT Kit

miScript SYBR® Green PCR Kit

miScript miRNA PCR Arrays

miScript miRNA QC PCR Array

For SYBR Green-based, real-time PCR profiling of microRNAs using pathway-focused arrays and miRNome arrays

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Contents

Kit Contents

miScript II RT Kit	**(12)**	**(50)**
Catalog no.	**218160**	**218161**
Number of standard reactions*	**12**	**50**
miScript Reverse Transcriptase Mix	24 µl	100 µl
10x miScript Nucleics Mix	50 µl	200 µl
5x miScript HiSpec Buffer	100 µl	400 µl
5x miScript HiFlex Buffer	100 µl	400 µl
RNase-Free Water	1.9 ml	1.9 ml
Quick-Start Protocol	1	1

* A standard reaction is 20 µl in volume with 10 pg–2 µg total RNA (when using miScript HiSpec Buffer) or 10 pg–1 µg total RNA (when using miScript HiFlex Buffer).

miScript SYBR Green PCR Kit	**(200)**	**(1000)**
Catalog no.	**218073**	**218075**
Number of 50 µl reactions	**200**	**1000**
2x QuantiTect® SYBR Green PCR Master Mix, containing: ■ HotStarTaq® DNA Polymerase ■ QuantiTect SYBR Green PCR Buffer ■ dNTP mix, including dUTP ■ SYBR Green I ■ ROX™ passive reference dye ■ 5 mM $MgCl_2$	3 x 1.7 ml	25 ml
10x miScript Universal Primer	1 ml	5 x 1 ml
RNase-Free Water	2 x 2 ml	20 ml
Quick-Start Protocol	2	2

Pathway-Focused miScript miRNA PCR Array

Catalog no.	**Varies**						
Format	**A**	**C**	**D**	**E**	**F**	**G**	**R**
96-well plate containing dried assays	2, 12, or 24	2, 12, or 24	2, 12, or 24	–	2, 12, or 24	–	–
384-well plate containing dried assays	–	–	–	4	–	4	–
Rotor-Disc® 100 containing dried assays	–	–	–	–	–	–	2, 12, or 24
Optical Thin-Wall 8-Cap Strips (12 per plate)	24, 144, or 288	–	24, 144, or 288	–	–	–	–
Optical Adhesive Film (1 per plate)	–	2, 12, or 24	–	4	2, 12, or 24	4	–
384EZLoad Covers (1 set of 4 per plate)	–	–	–	4 sets	–	4 sets	–
Rotor-Disc Heat Sealing Film (1 per Rotor-Disc)	–	–	–	–	–	–	2, 12, or 24

Human/Mouse/Rat/Dog miRNome miScript miRNA PCR Array

Catalog no.	**Varies**						
Format	**A**	**C**	**D**	**E**	**F**	**G**	**R**
96-well plate containing dried assays	Varies	Varies	Varies	–	Varies	–	–
384-well plate containing dried assays	–	–	–	Varies	–	Varies	–
Rotor-Disc 100 containing dried assays	–	–	–	–	–	–	Varies
Optical Thin-Wall 8-Cap Strips (12 per plate)	Varies	–	Varies	–	–	–	–
Optical Adhesive Film (1 per plate)	–	Varies	–	Varies	Varies	Varies	–
Rotor-Disc Heat Sealing Film (1 per Rotor-Disc)	–	–	–	–	–	–	Varies

miScript miRNA QC PCR Array

Catalog no.	**Varies**						
Format	**A**	**C**	**D**	**E**	**F**	**G**	**R**
96-well plate containing dried assays	1	1	1	–	1	–	–
384-well plate containing dried assays	–	–	–	1	–	1	–
Rotor-Disc 100 containing dried assays	–	–	–	–	–	–	1
Optical Thin-Wall 8-Cap Strips	12	–	12	–	–	–	–
Optical Adhesive Film	–	1	–	1	1	1	–
Rotor-Disc Heat Sealing Film (1 per Rotor-Disc)	–	–	–	–	–	–	1

Cyclers for use with array formats

Format	Suitable real-time cyclers	Plate
A	Applied Biosystems® models 5700, 7000, 7300, 7500, 7700, 7900HT, ViiA™ 7 (96-well block); Bio-Rad® models iCycler®, iQ™5, MyiQ™, MyiQ2; Bio-Rad/MJ Research Chromo4™; Eppendorf® Mastercycler® ep realplex models 2, 2S, 4, 4S; Stratagene® models Mx3005P®, Mx3000P®; Takara TP-800	96-well
C	Applied Biosystems models 7500 (Fast block), 7900HT (Fast block), StepOnePlus™, ViiA 7 (Fast block)	96-well
D	Bio-Rad CFX96™; Bio-Rad/MJ Research models DNA Engine Opticon®, DNA Engine Opticon 2; Stratagene Mx4000®	96-well
E	Applied Biosystems models 7900HT (384-well block), ViiA 7 (384-well block); Bio-Rad CFX384™	384-well
F	Roche® LightCycler® 480 (96-well block)	96-well
G	Roche LightCycler 480 (384-well block)	384-well
R	Rotor-Gene® Q; Rotor-Gene 6000; other Rotor-Gene cyclers	Rotor-Disc 100

Shipping and Storage

The miScript II RT Kit and miScript SYBR Green PCR Kit are shipped on dry ice. The kits, including all reagents and buffers, should be stored immediately upon receipt at –20°C in a constant-temperature freezer.

miScript miRNA PCR Arrays and miScript miRNA QC PCR Arrays are shipped at ambient temperature, on ice, or on dry ice depending on the destination and accompanying products. Upon receipt, store at –20 °C. If stored under these conditions, miScript miRNA PCR Arrays and miScript miRNA QC PCR Arrays are stable for 6 months after receipt.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of miScript II RT Kit and miScript SYBR Green PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

The miScript II RT Kit, miScript SYBR Green PCR Kit, miScript miRNA PCR Arrays and miScript miRNA QC PCR Array are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the miScript II RT Kit, miScript SYBR Green PCR Kit, miScript miRNA PCR Arrays, miScript miRNA QC PCR Array, or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/Support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

24-hour emergency information

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Poison Information Center Mainz, Germany

Tel: +49-6131-19240

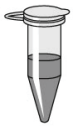
Introduction

The miScript PCR System consists of the miScript II RT Kit, miScript SYBR Green PCR Kit, miScript Primer Assay, miScript miRNA PCR Array, and miScript miRNA PCR Array data analysis tool. The miScript PCR System allows sensitive and specific detection and quantification of microRNA (miRNA). The miScript PCR System uses total RNA that contains miRNA as the starting material for cDNA synthesis, and separate enrichment of small RNA is not needed. A single cDNA preparation can be used with a miScript miRNA PCR Array to rapidly profile the expression of mature miRNAs.

miScript miRNA PCR Arrays are mature miRNA-specific forward primers (miScript Primer Assays) that have been arrayed in biologically relevant pathway-focused and whole miRNome panels. These PCR arrays are provided in ready-to-use 384-well plate, 96-well plate, and 100-well Rotor-Disc formats. miScript miRNA PCR Arrays, which are available for several species, provide guaranteed high performance and are fully customizable. Each assay in a miScript miRNA PCR Array has been verified to ensure sensitive and specific detection of mature miRNA by real-time PCR. A free, Web-based miScript miRNA PCR Array data analysis tool simplifies the analysis of real-time PCR data. Once raw threshold cycle (C_T) data has been uploaded, the tool automatically performs all fold-change calculations using the $\Delta\Delta C_T$ method of relative quantification, and presents the results in several formats. Mature miRNome expression profiling is now within reach of every laboratory because of the ease, convenience, and consistent performance of miScript miRNA PCR Arrays. miScript miRNA PCR Arrays are at the forefront of real-time PCR-based mature miRNA profiling tools.

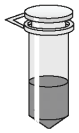
miScript miRNA PCR Array workflow

Prepare reverse-transcription reaction

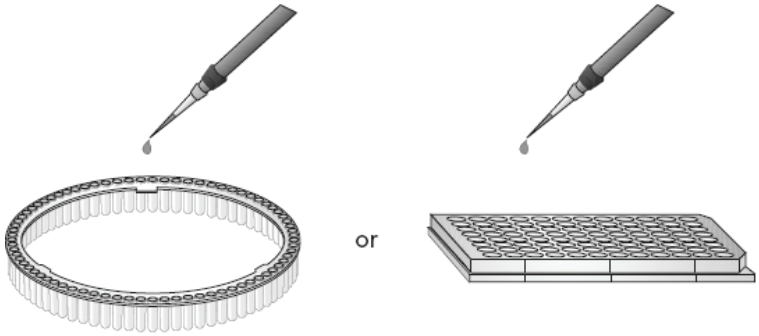


Incubate at 37°C for 60 min, then at 95°C for 5 min

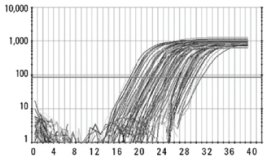
Prepare PCR mix



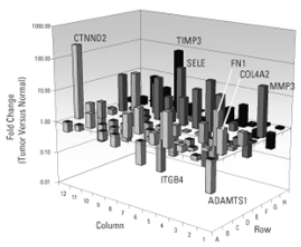
Add PCR mix to miScript miRNA PCR Array

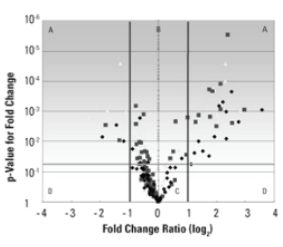


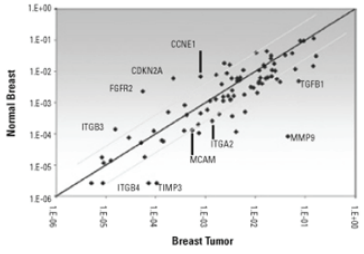
Perform real-time PCR



Analyze results using miScript miRNA PCR Array data analysis tool







miScript II RT Kit

The expanded miScript II RT Kit includes miScript Reverse Transcriptase Mix, 10x miScript Nucleics Mix, 5x miScript HiSpec Buffer, and 5x miScript HiFlex Buffer. miScript Reverse Transcriptase Mix is an optimized blend of poly(A) polymerase and reverse transcriptase. 10x miScript Nucleics Mix contains dNTPs, rATP, oligo-dT primers, and an internal synthetic RNA control (miRNA reverse transcription control [miRTC]) that is used to assess reverse transcription performance during profiling experiments with miScript miRNA PCR Arrays. Two buffers, 5x miScript HiSpec Buffer and 5x miScript HiFlex Buffer, are provided in the miScript II RT Kit to meet the distinctive needs of miRNA quantification studies using real-time PCR (Figure 1). The unique, patent-pending formulation of 5x miScript HiSpec Buffer facilitates the selective conversion of mature miRNAs into cDNA, which can then be used for miRNA quantification with either miScript miRNA PCR Arrays or miScript Primer Assays. For protocols using miScript Primer Assays, refer to the *miScript PCR System Handbook*. 5x miScript HiFlex Buffer promotes conversion of all RNA species (mature miRNA, precursor miRNA, other noncoding RNA, and mRNA) into cDNA, and this cDNA can then be used to quantify each RNA species (using appropriate primer assays). For protocols using miScript HiFlex Buffer, refer to the *miScript PCR System Handbook*.

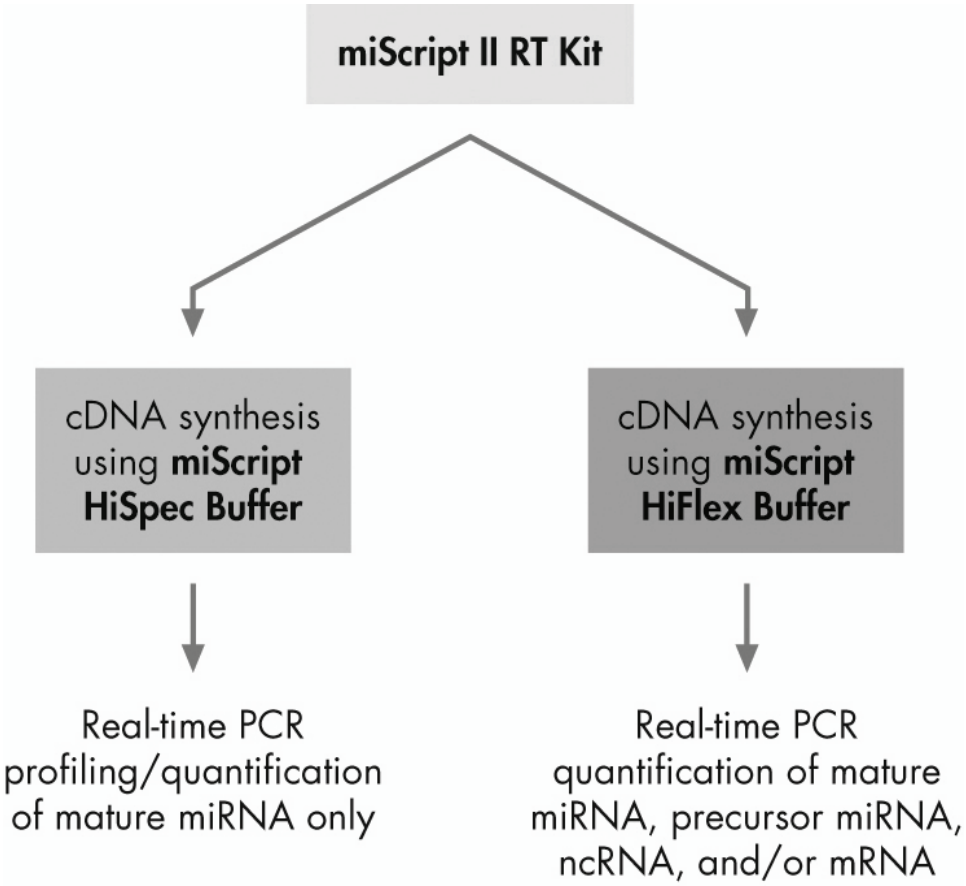


Figure 1. Mature miRNA, precursor miRNA, other noncoding RNA, and mRNA detection. Two buffers are provided with the miScript II RT Kit. Use miScript HiSpec Buffer for cDNA synthesis to enable either mature miRNA profiling (using miScript miRNA PCR Arrays) or mature miRNA quantification (using individual miScript Primer Assays). For protocols using miScript HiSpec Buffer in combination with miScript Primer Assays, refer to the *miScript PCR System Handbook*. Use miScript HiFlex Buffer for cDNA synthesis to enable quantification of mature miRNA, precursor miRNA, other noncoding RNA (ncRNA), and/or mRNA from the same cDNA (using appropriate primer assays). For protocols using miScript HiFlex Buffer, refer to the *miScript PCR System Handbook*.

Principle and procedure

Mature miRNAs are naturally occurring, 22-nucleotide, noncoding RNAs that mediate posttranscriptional gene regulation. Unlike mRNAs, miRNAs are not polyadenylated in nature.

Reverse-transcription in miScript HiSpec Buffer

When reverse transcription reactions are performed in miScript HiSpec Buffer, mature miRNAs and certain small nucleolar RNAs and small nuclear RNAs (snoRNAs and snRNAs, see "Controls in miScript miRNA PCR Arrays and miScript miRNA QC PCR Arrays", page 22) are selectively converted into cDNA. Mature miRNAs are polyadenylated by poly(A) polymerase and reverse transcribed into cDNA using oligo-dT primers (Figure 2). Polyadenylation and

reverse transcription are performed in parallel in the same tube. The oligo-dT primers have a 3' degenerate anchor and a universal tag sequence on the 5' end, allowing amplification of mature miRNA in the real-time PCR step. miScript miRNA PCR Arrays, used in combination with the miScript SYBR Green PCR Kit, enable quantification of mature miRNA by real-time PCR. The combination of polyadenylation and the universal tag addition ensures that miScript miRNA PCR Arrays do not detect genomic DNA.

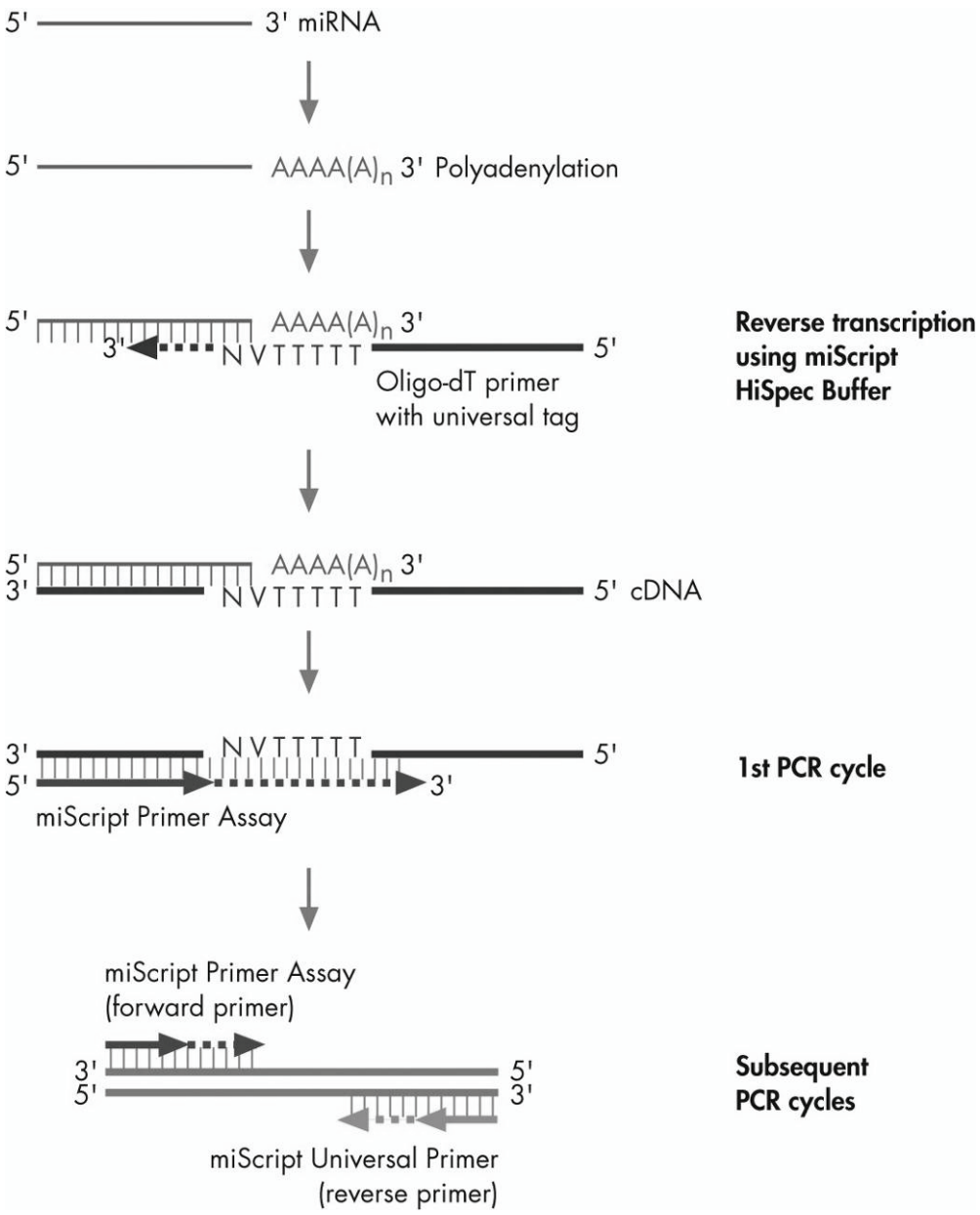


Figure 2. Conversion of mature miRNAs into cDNA and subsequent detection. In a reverse-transcription reaction using miScript HiSpec Buffer, mature miRNAs are polyadenylated by poly(A) polymerase and subsequently converted into cDNA by reverse transcriptase with oligo-dT priming. The cDNA is then used for real-time PCR profiling of mature miRNA expression (using a miScript miRNA PCR Array and the miScript Universal Primer).

Real-time PCR for profiling mature miRNA expression

cDNA prepared in a reverse transcription reaction using miScript HiSpec Buffer serves as the template for real-time PCR analysis using a miScript miRNA PCR Array (which contains miRNA-specific miScript Primer Assays) and the miScript SYBR Green Kit, which contains the miScript Universal Primer (reverse primer) and QuantiTect SYBR Green PCR Master Mix. To profile mature miRNA expression, a premix of cDNA, miScript Universal Primer, QuantiTect SYBR Green PCR Master Mix, and RNase-free water is added to a miScript miRNA PCR Array.

miScript miRNA PCR Array plate layout

miScript miRNA PCR Arrays are available in 96-well, 384-well, and Rotor-Disc 100 formats (Figures 3–6). Each array contains several control assays. The purpose of each control is described on page 22.

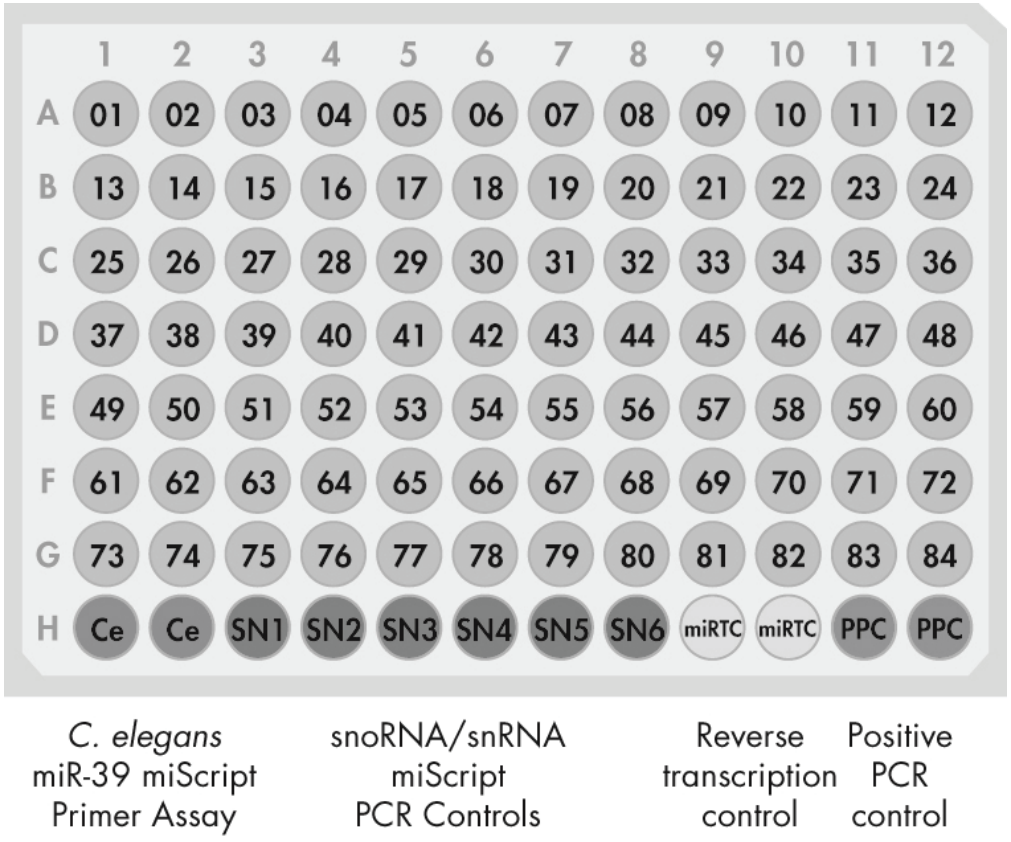


Figure 3. Pathway-Focused or miRNome miScript miRNA PCR Array layout for plate formats A, C, D, F. Wells A1 to G12 (**1**–**84**) each contain a miScript Primer Assay for a pathway/disease/functionally related mature miRNA. Wells H1 and H2 contain replicate *C. elegans* miR-39 miScript Primer Assays that can be used as an alternative normalizer for array data (**Ce**). Wells H3 to H8 each contain an assay for a different snoRNA/snRNA that can be used as a normalization control for the array data (**SN1**=SNORD61 assay, **SN2**=SNORD68 assay, **SN3**=SNORD72 assay, **SN4**=SNORD95 assay, **SN5**=SNORD96A assay, **SN6**=RNU6B/RNU6-2 assay). Wells H9 and H10 contain replicate reverse transcription controls (**miRTC**). Wells H11 and H12 contain replicate positive PCR controls (**PPC**).

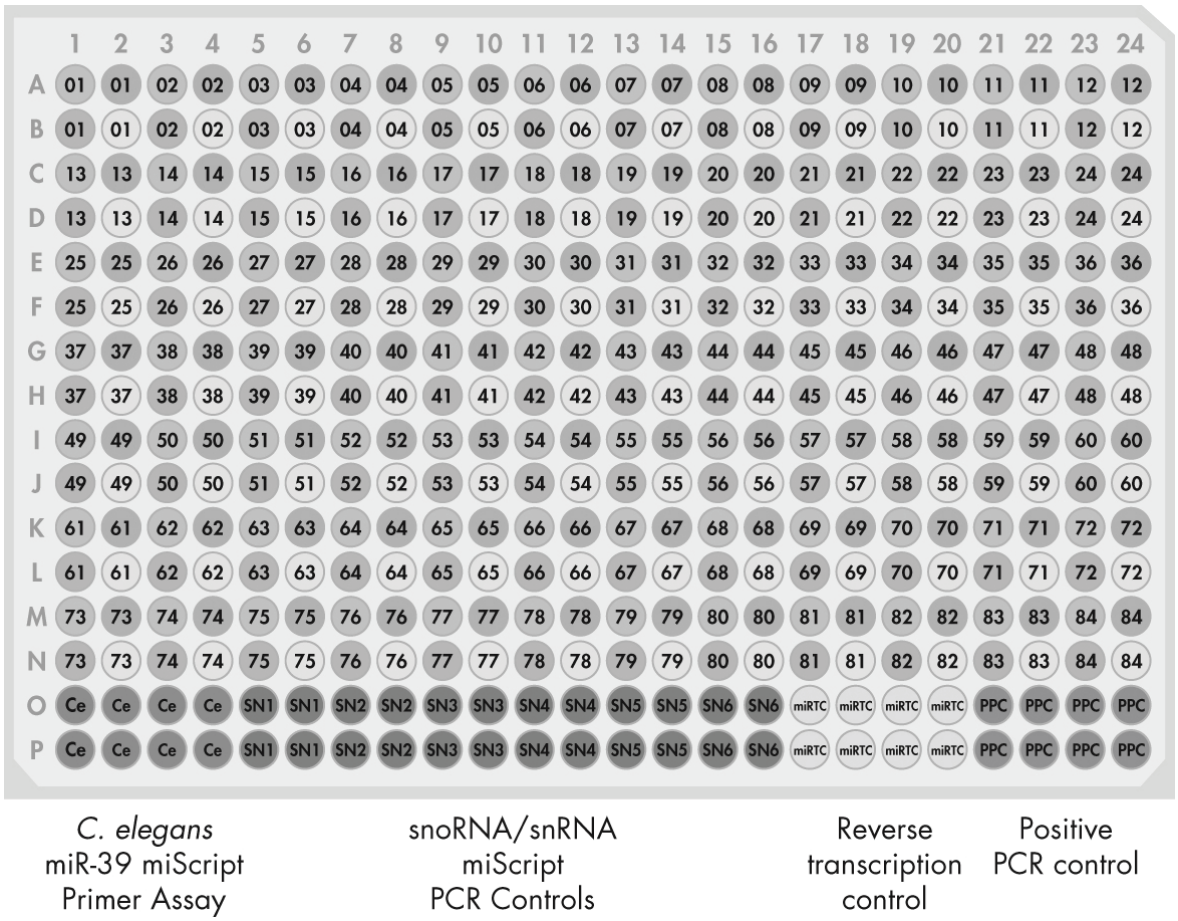


Figure 4. Pathway-Focused miScript miRNA PCR Array layout for plate formats E, G. Pathway-Focused miScript miRNA PCR Arrays in formats E and G include 4 replicates of the same assays as provided in the 96-well format shown in Figure 3.

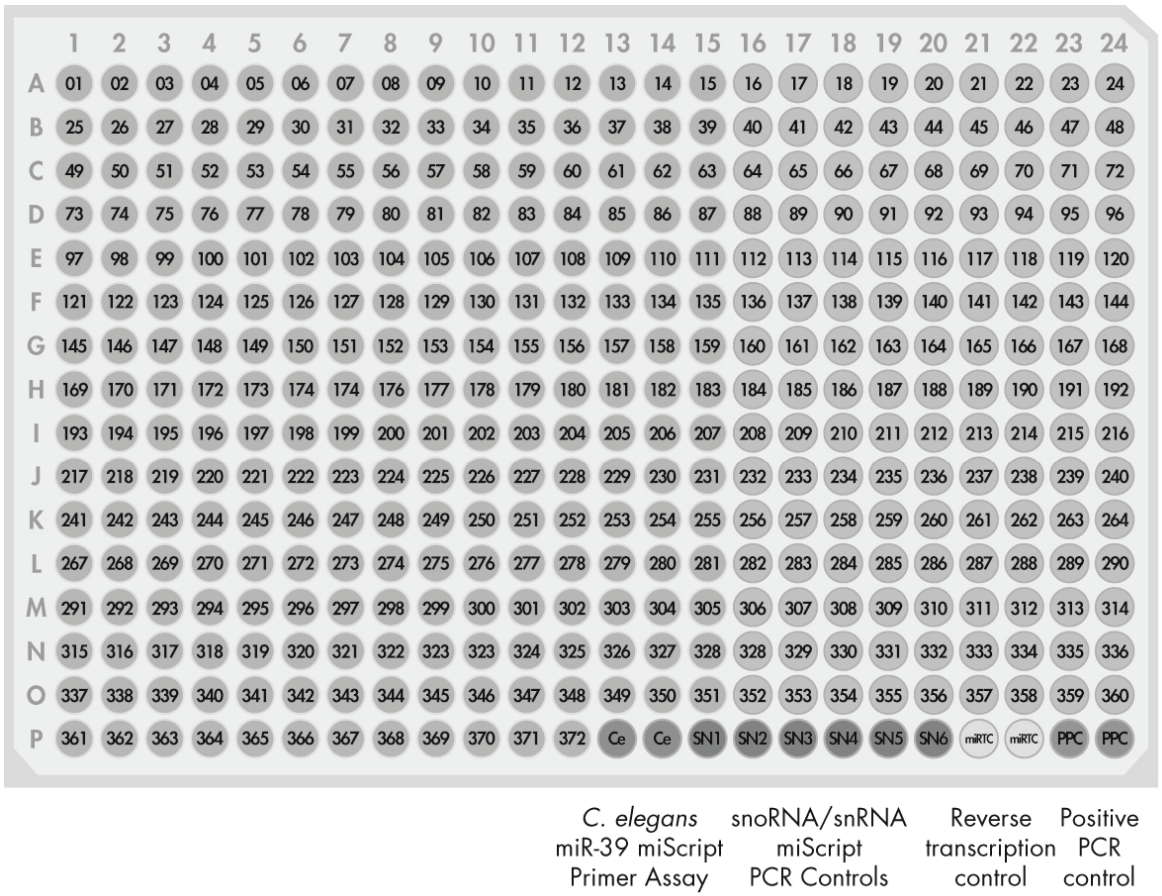


Figure 5. miRNome miScript miRNA PCR Array layout for plate formats E, G. Wells A1 to P12 (**1**–**372**) each contain a miScript Primer Assay for a pathway/disease/ functionally related mature miRNA. Wells P13 and P14 contain replicate C. *elegans* miR-39 miScript Primer Assays that can be used as an alternative normalizer for array data (**Ce**). Wells P15 to P20 each contain an assay for a different snoRNA/snRNA that can be used as a normalization control for the array data (**SN1**=SNORD61 assay, **SN2**=SNORD68 assay, **SN3**=SNORD72 assay, **SN4**=SNORD95 assay, **SN5**=SNORD96A assay, **SN6**=RNU6B/RNU6-2 assay). Wells P21 and P22 contain replicate reverse transcription controls (**miRTC**). Wells P23 and P24 contain replicate positive PCR controls (**PPC**).

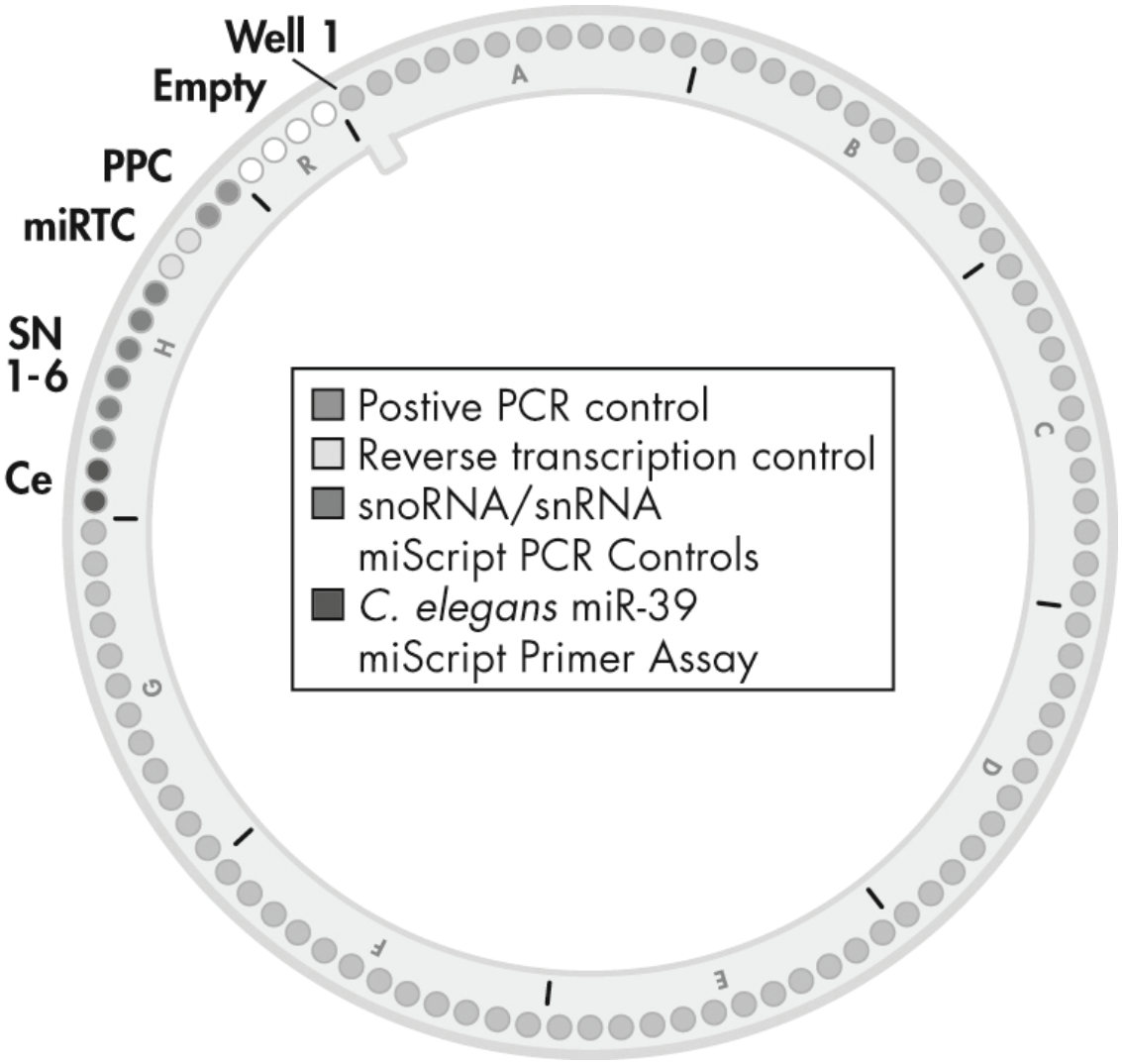


Figure 6. miScript miRNA PCR Array layout for Rotor-Disc format R. Wells 1 to 84 each contain a miScript Primer Assay for a pathway/disease/functionally related gene. Wells 85 and 86 contain replicate *C. elegans* miR-39 miScript Primer Assays that can be used as an alternative normalizer for array data (**Ce**). Wells 87 to 92 each contain an assay for a different snoRNA/snRNA that can be used as a normalization control for the array data (**SN1**=SNORD61 assay, **SN2**=SNORD68 assay, **SN3**=SNORD72 assay, **SN4**=SNORD95 assay, **SN5**=SNORD96A assay, **SN6**=RNU6B/RNU6-2 assay). Wells 93 and 94 contain replicate reverse transcription controls (**miRTC**). Wells 95 and 96 contain replicate positive PCR controls (**PPC**). Wells 97–100 are empty.

miScript miRNA QC PCR Array plate layout

miScript miRNA QC PCR Arrays enable assessment of the quality of multiple cDNA samples using real-time PCR. miScript miRNA QC PCR Arrays are available in 96-well, 384-well, and Rotor-Disc 100 formats (Figures 7–9). Each miScript miRNA QC PCR Array contains replicates of the control assays found on the miScript miRNA PCR Array. The purpose of each control is described on page 22. The 96-well plate and Rotor-Disc 100 formats allow quality control of up to 8 cDNA samples. The 384-well format allows quality control of up to 32 cDNA samples.

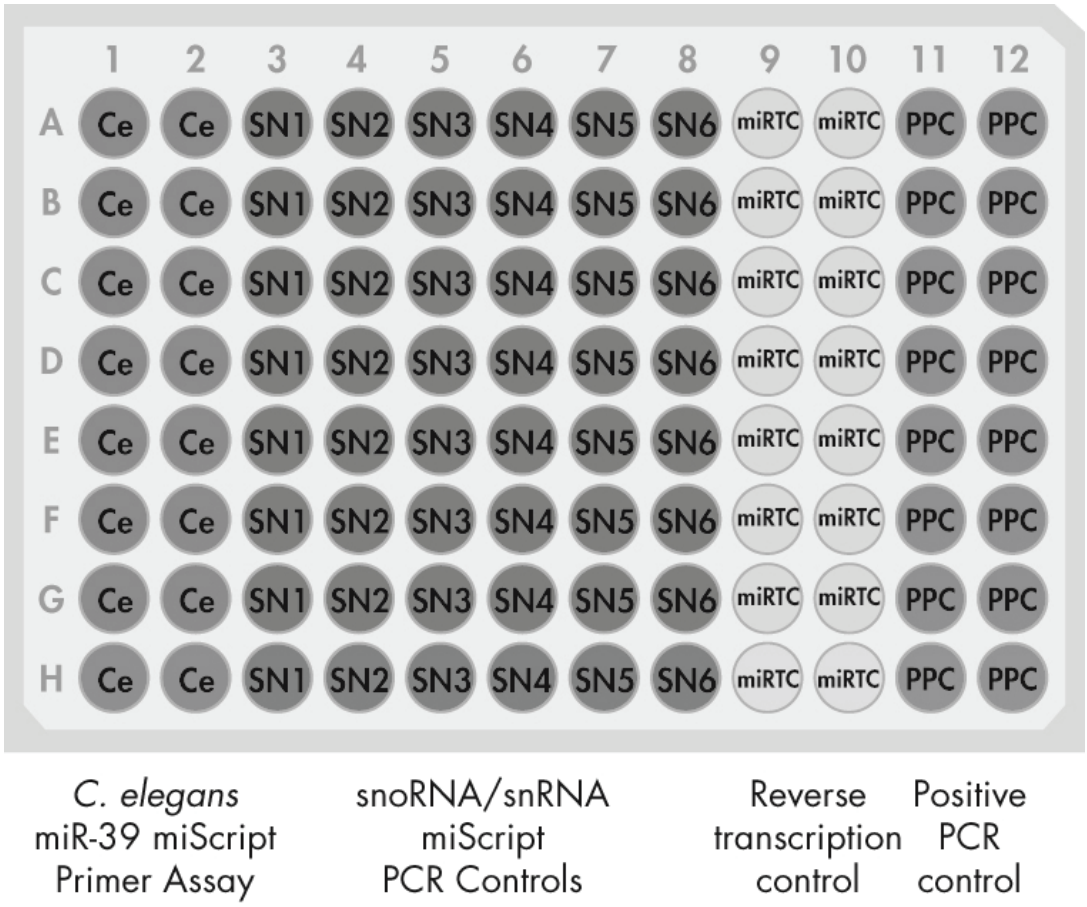


Figure 7. miScript miRNA QC PCR Array layout for plate formats A, C, D, F. Wells 1 and 2 of each row contain replicate *C. elegans* miR-39 miScript Primer Assays (**Ce**). Wells 3 to 8 of each row each contain an assay for a different snoRNA/snRNA (**SN1**=SNORD61 assay, **SN2**=SNORD68 assay, **SN3**=SNORD72 assay, **SN4**=SNORD95 assay, **SN5**=SNORD96A assay, **SN6**=RNU6B/RNU6-2 assay). Wells 9 and 10 of each row contain replicate reverse transcription controls (**miRTC**). Wells 11 and 12 of each row contain replicate positive PCR controls (**PPC**). These formats enable the quality assessment of up to 8 cDNA samples.

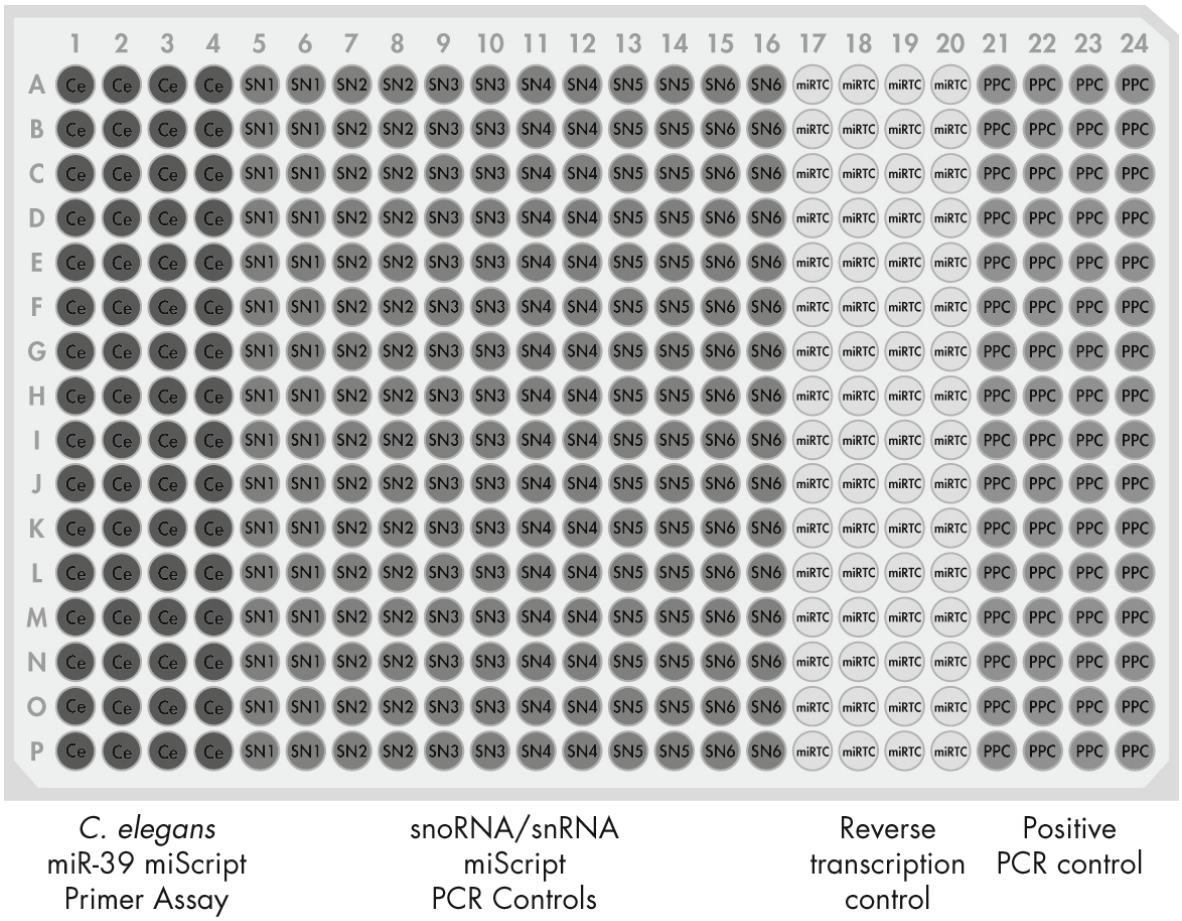


Figure 8. miScript miRNA QC PCR Array layout for plate formats E and G. Wells 1 to 4 of each row contain replicate *C. elegans* miR-39 miScript Primer Assays (**Ce**). Wells 5 to 16 of each row each contain an assay for a different snoRNA/snRNA (**SN1**=SNORD61 assay, **SN2**=SNORD68 assay, **SN3**=SNORD72 assay, **SN4**=SNORD95 assay, **SN5**=SNORD96A assay, **SN6**=RNU6B/RNU6-2 assay). Wells 17 to 20 of each row contain replicate reverse transcription controls (**miRTC**). Wells 21 to 24 of each row contain replicate positive PCR controls (**PPC**). These formats enable the quality assessment of up to 32 cDNA samples.

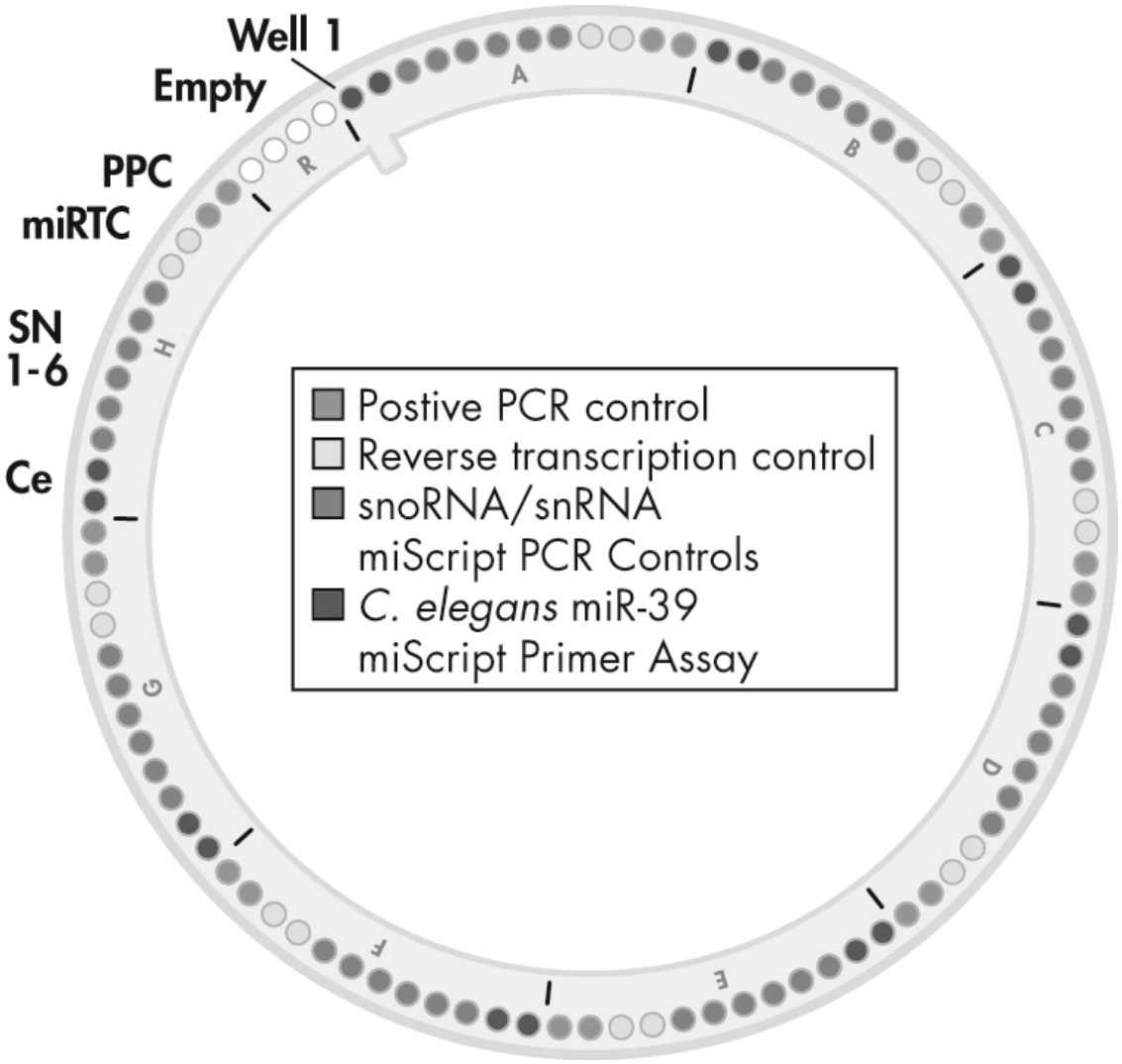


Figure 9. miScript miRNA QC PCR Array layout for Rotor-Disc format R. Wells 1 and 2 contain replicate *C. elegans* miR-39 miScript Primer Assays (**Ce**). Wells 3 to 8 each contain an assay for a different snoRNA/snRNA (**SN1**=SNORD61 assay, **SN2**=SNORD68 assay, **SN3**=SNORD72 assay, **SN4**=SNORD95 assay, **SN5**=SNORD96A assay, **SN6**=RNU6B/RNU6-2 assay). Wells 9 and 10 contain replicate reverse transcription controls (**miRTC**). Wells 11 and 12 contain replicate positive PCR controls (**PPC**). This pattern is repeated 7 additional times from wells 13 to 96. Wells 97 to 100 are empty. This format enables the quality assessment of up to 8 cDNA samples.

Controls in miScript miRNA PCR Arrays and miScript miRNA QC PCR Arrays

The final 12 wells of each miScript miRNA PCR Array contain controls. The purpose of each control is detailed below and in Table 1.

Table 1. Controls in miScript PCR Arrays

Control	Purpose
C. elegans miR-39 miScript Primer Assay	Alternative data normalization using exogenously spiked Syn-cel-miR-39 miScript miRNA Mimic
6 snoRNA/snRNA miScript Primer Assays (miScript PCR Controls)	Data normalization using the $\Delta\Delta C_T$ method of relative quantification
miRNA reverse transcription control (miRTC)	Assessment of reverse transcription performance
Positive PCR control (PPC)	Assessment of PCR performance

The miScript Primer Assay for *C. elegans* miR-39 detects Syn-cel-miR-39 miScript miRNA Mimic (cat. no. MSY0000010). This mimic can be added to samples, particularly serum or plasma samples, to control for variations during the preparation of total RNA and subsequent steps. After purification, real-time RT-PCR detection of the *C. elegans* miScript miRNA Mimic can be performed and these results can then be used for normalization of real-time RT-PCR results for endogenous miRNAs in the sample. For more information on the use of Syn-cel-miR-39 miScript miRNA Mimic, see the *QIAGEN Supplementary Protocol: Purification of total RNA, including small RNAs, from serum or plasma using the miRNeasy Mini Kit* at www.qiagen.com/miRNeasyMiniResources.

For accurate and reproducible results in miRNA quantification by real-time PCR, it is necessary to normalize the amount of target miRNA by using a suitable endogenous reference RNA. This approach is known as relative quantification. Normalization corrects for factors that could otherwise lead to inaccurate quantification. These factors include variation in quantity of input RNA, possible RNA degradation or presence of inhibitors in the RNA samples, and differences in sample handling. Normalization also allows results from different experiments and samples to be compared directly. miScript PCR Controls are primers designed to quantify a panel of 5 snoRNAs (SNORD61, SNORD68, SNORD72, SNORD95, and SNORD96A) and the snRNA RNU6B (RNU6-2). These controls take into consideration sequence homologies in human, mouse, rat, and dog so that the same controls can be used for all 4 species. In addition, these small RNAs have been verified to have relatively stable expression levels across tissues and cell types. As a result, miScript PCR Controls

serve as normalization controls for relative quantification using the miScript PCR System. All the controls have amplification efficiencies close to 100%. For more information and to view data, visit www.qiagen.com/miRNAControls.

The miRNA reverse transcription control (miRTC) is an assay that assesses the performance of a reverse transcription reaction using the miScript II RT Kit by detecting template synthesized from the kit's built-in control RNA. This control monitors for any variables that may inhibit the reverse transcription reaction.

The positive PCR control (PPC) wells contain a predispensed artificial DNA sequence and the assay that detects it. This control monitors for any variables that may inhibit the PCR reaction.

Data analysis

Free data analysis software for each miScript miRNA PCR Array is available at http://pcrdataanalysis.sabiosciences.com/mirna. At this Web page, both the miScript miRNA PCR Array Web-based software and the miScript miRNA PCR Array Data Analysis Excel® Template can be accessed. Both tools will automatically perform quantification using the $\Delta\Delta C_T$ method of relative quantification and interpretation of the control assays (for more details, see page 35). Results are presented in a tabular format, a scatter plot, a three-dimensional profile, and a volcano plot (when replicates are included).

Template RNA requirements

Total RNA containing miRNA is the required starting material for miScript miRNA PCR Arrays. It is not necessary to enrich for small RNA. QIAGEN provides a range of solutions for purification of total RNA including miRNA (Table 2).

Table 2. Kits for purification of RNA including miRNA

Kit	Cat. no.	Starting material
miRNeasy Mini Kit	217004	Animal/human tissues, cells, and serum/plasma*
miRNeasy 96 Kit	217061	Animal/human tissues and cells
miRNeasy FFPE Kit	217504	Formalin-fixed, paraffin-embedded (FFPE) tissue samples
PAXgene® Tissue miRNA Kit	766134	Animal/human tissues that have been fixed and stabilized in PAXgene Tissue Containers
PAXgene Blood miRNA Kit	763134	Human blood that has been stabilized in PAXgene Blood RNA Tubes

* miRNA can be purified from serum or plasma samples using the *QIAGEN Supplementary Protocol: Purification of total RNA, including small RNAs, from serum or plasma using the miRNeasy Mini Kit*. For more information, visit www.qiagen.com/miRNeasyMiniResources.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

For reverse transcription

- Thin-walled, DNase-free, RNase-free PCR tubes (for 20 µl reactions)
- Ice
- Thermal cycler, heating blocks, or water baths (capable of reaching 95°C)
- Microcentrifuge

For quantitative, real-time PCR

- Real-time PCR cycler; the table on page 7 shows the appropriate real-time cycler for each array format
- Multichannel pipettor
- Nuclease-free pipet tips and tubes

Protocol: Reverse Transcription for Quantitative, Real-Time PCR

Important points before starting

- The miScript II RT Kit includes 2 buffers: 5x miScript HiSpec Buffer and 5x miScript HiFlex Buffer. **Only 5x miScript HiSpec Buffer should be used to prepare cDNA for mature miRNA profiling** using Pathway-Focused or miRNome miScript miRNA PCR Arrays. (For protocols using miScript HiFlex Buffer in reverse transcription reactions for parallel quantification of mature miRNA, small noncoding RNA, precursor miRNA, and mRNA, refer to the *miScript PCR System Handbook*.)
- Total RNA containing miRNA should be used as starting material. For RNA purification recommendations, see page 24. This protocol is for use with up to 2 µg RNA. If using higher RNA amounts, scale up the reaction linearly. Recommended starting amounts are shown in Table 3. If working with RNA for the first time, read Appendix B (page 51).
- Set up all reactions on ice to minimize the risk of RNA degradation.
- Do not vortex template RNA or any of the components of the miScript II RT Kit.

Table 3. Recommended RNA starting amounts and buffers for reverse-transcription reactions

PCR application	Assay	Buffer	Recommended RNA input*†
Pathway profiling of mature miRNA	Pathway-Focused miScript miRNA PCR Arrays	5x miScript HiSpec Buffer	125–250 ng per RNA sample
Whole miRNome profiling of mature miRNA	miRNome miScript miRNA PCR Arrays	5x miScript HiSpec Buffer	250–500 ng per 384-well plate or per 4 x 96-well plates/ Rotor-Discs (the number of plates provided in a miRNome miScript miRNA PCR Array varies depending on the species of interest)‡

* If the RNA sample is not limiting, use the upper amount of the recommended range.

† These recommended RNA starting amounts result in 0.5–1 ng cDNA per array well.

‡ Depending on the RNA starting amount, a single reverse transcription reaction can provide sufficient cDNA for 8 x 384-well plates or 32 x 96-well plates/Rotor-Discs.

Procedure

1. **Thaw template RNA on ice. Thaw RNase-free water, 10x miScript Nucleics Mix and 5x miScript HiSpec Buffer at room temperature (15–25°C).**

   Mix each solution by flicking the tubes. Centrifuge briefly to collect residual liquid from the sides of the tubes and then store on ice.

2. **Prepare the reverse-transcription reaction on ice according to Table 4.**

   Gently mix and then store on ice. The reverse-transcription master mix contains all components required for first-strand cDNA synthesis except template RNA.

   Note: miScript Reverse Transcriptase Mix should be removed from the –20°C freezer just before preparation of the master mix, gently mixed, and placed on ice. It should be returned to the freezer immediately after use.

   Note: If setting up more than one reaction, prepare a volume of master mix 10% greater than that required for the total number of reactions to be performed.

Table 4. Reverse-transcription reaction components

Component	Volume/reaction
5x miScript HiSpec Buffer	4 µl
10x miScript Nucleics Mix	2 µl
RNase-free water	Variable
miScript Reverse Transcriptase Mix	2 µl
Template RNA (added in step 3)	Variable (see Table 3 for recommendations)*
Total volume	**20 µl**

* If using RNA from serum or plasma samples prepared using the *QIAGEN Supplementary Protocol: Purification of total RNA, including small RNAs, from serum or plasma using the miRNeasy Mini Kit*, we recommend using 5 µl of the RNA preparation as a starting point with Pathway-Focused miScript miRNA PCR Arrays.

3. **Add template RNA to each tube containing reverse-transcription master mix. Gently mix, briefly centrifuge, and then store on ice.**
4. **Incubate for 60 min at 37°C.**
5. **Incubate for 5 min at 95°C to inactivate miScript Reverse Transcriptase Mix and place on ice.**
6. **Dilute the cDNA in RNase-free water according to Table 5, and proceed with real-time PCR immediately.**

   If you wish to store the reverse-transcription reactions prior to real-time PCR, transfer the undiluted cDNA to a –20°C freezer, or dispense the diluted cDNA into 110 µl aliquots and transfer them to a –20°C freezer.

Table 5. cDNA dilution prior to PCR

PCR application	Assay	Reaction dilution
Pathway profiling	Pathway-Focused miScript miRNA PCR Arrays	Add 200 µl RNase-free water to each 20 µl reverse-transcription reaction
Whole miRNome profiling	miRNome miScript miRNA PCR Arrays	Dilution depends on the number of plates/Rotor-Discs in the miRNome miScript miRNA PCR Array: For 1 x 384-well plate or 4 x 96-well plates/Rotor-Discs: add 90 µl RNase-free water to the 20 µl reverse-transcription reaction For 2 x 384-well plate or 8 x 96-well plates/Rotor-Discs: add 200 µl RNase-free water to the 20 µl reverse-transcription reaction For 3 x 384-well plate or 12 x 96-well plates/Rotor-Discs: add 310 µl RNase-free water to the 20 µl reverse-transcription reaction For 4 x 384-well plate or 16 x 96-well plates/Rotor-Discs: add 420 µl RNase-free water to the 20 µl reverse-transcription reaction

Protocol: Real-Time PCR for Mature miRNA Expression Profiling

cDNA prepared using the miScript II RT Kit with miScript HiSpec Buffer is the appropriate starting material for this protocol. This protocol enables real-time PCR profiling of mature miRNA using miScript miRNA PCR Arrays in combination with the miScript SYBR Green PCR Kit, which contains the miScript Universal Primer (reverse primer) and QuantiTect SYBR Green PCR Master Mix.

Important points before starting

- The PCR must start with an **initial incubation step of 15 minutes at 95°C** to activate HotStarTaq DNA Polymerase (included in 2x QuantiTect SYBR Green PCR Master Mix).
- Only cDNA template prepared using miScript HiSpec Buffer should be used with this protocol.
- Ensure that the 20 µl cDNA synthesis reaction has been diluted appropriately. See Table 5 for recommendations.
- Do not vortex template cDNA or any of the components of the miScript SYBR Green PCR Kit.
- The standard miScript miRNA PCR Array reaction volumes are 10 µl per well for a 384-well plate, 25 µl per well for a 96-well plate, and 20 µl per well for a 100-well Rotor-Disc.
- The miScript SYBR Green PCR Kit (200) provides enough reagents for at least 2 x 384-well arrays, 3 x 96-well arrays, or 4 x Rotor-Disc 100 arrays. The miScript SYBR Green PCR Kit (1000) provides enough reagents for at least 11 x 384-well arrays, 18 x 96-well arrays, or 22 x Rotor-Disc 100 arrays.
- If using the iCycler iQ, iQ5, or MyiQ, well factors must be collected at the beginning of each experiment. Well factors are used to compensate for any system or pipetting nonuniformity. For details, refer to the user manual supplied with the instrument or *Technical Information: Using QuantiTect SYBR Green Kits on Bio-Rad cyclers* available at www.qiagen.com.
- The miScript miRNA QC PCR Array can be used to assess the quality of cDNA samples prior to running a miScript miRNA PCR Array (see protocol, page 38).

Procedure

1. **Thaw 2x QuantiTect SYBR Green PCR Master Mix, 10x miScript Universal Primer, template cDNA, and RNase-free water at room temperature (15–25°C). Mix the individual solutions.**

2. **Prepare a reaction mix according to either Table 6 (for a Pathway-Focused miScript miRNA PCR Array) or Table 7 (for a miRNome miScript miRNA PCR Array). Mix thoroughly but gently.**

   Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the real-time cycler.

Table 6. Reaction mix for Pathway-Focused miScript miRNA PCR Arrays*

Array format: Component	384-well (4 x 96) Formats E, G†	96-well Formats A, C, D, F	Rotor-Disc 100 Format R
2x QuantiTect SYBR Green PCR Master Mix‡	550 µl	1375 µl	1100 µl
10x miScript Universal Primer	110 µl	275 µl	220 µl
RNase-free water	340 µl	1000 µl	780 µl
Template cDNA§	100 µl	100 µl	100 µl
Total volume	**1100 µl**	**2750 µl**	**2200 µl**

* These volumes provide a 10 µl per well reaction volume for a 384-well plate, 25 µl per well reaction volume for a 96-well plate, and 20 µl per well reaction volume for a 100-well Rotor-Disc.

† Volumes shown are sufficient for one cDNA template. In total, 4 cDNA templates can be analyzed on one 384-well Pathway-Focused miScript miRNA PCR Array because assays are arrayed in quadruplicate (see Figure 4, page 16).

‡ No optimization of the Mg^{2+} concentration is required. The final Mg^{2+} concentration provided by 2x QuantiTect SYBR Green PCR Master Mix gives optimal results.

§ Provides 0.5–1 ng cDNA per well.

Table 7. Reaction mix for miRNome miScript miRNA PCR Arrays*†

Array format: Component	384-well Formats E, G	96-well Formats A, C, D, F	Rotor-Disc 100 Format R
2x QuantiTect SYBR Green PCR Master Mix‡	2050 µl	1375 µl	1100 µl
10x miScript Universal Primer	410 µl	275 µl	220 µl
RNase-free water	1540 µl	1075 µl	855 µl
Template cDNA§	100 µl	25 µl	25 µl
Total volume	**4100 µl**	**2750 µl**	**2200 µl**

* Volumes are for a single plate or Rotor-Disc associated with a miRNome set. The number of plates in a miRNome set vary depending on the species. Scale up volumes according to the number of plates/Rotor-Discs to be run. If the miRNome set contains a plate/Rotor-Disc that is less than half full, scale down volumes accordingly.

† These volumes provide a 10 µl per well reaction volume for a 384-well plate, 25 µl per well reaction volume for a 96-well plate, and 20 µl per well reaction volume for a 100-well Rotor-Disc.

‡ No optimization of the Mg^{2+} concentration is required. The final Mg^{2+} concentration provided by 2x QuantiTect SYBR Green PCR Master Mix gives optimal results.

§ Provides 0.5–1 ng cDNA per well.

3. **Carefully remove the miScript miRNA PCR Array from its sealed bag.**
   Optional for 96-well and 384-well array formats: If the reaction mix is in a tube, transfer to a loading reservoir, such as the RT² PCR Array Loading Reservoir (cat. no. 338162).

4. **Add reaction mix to each well of the miScript miRNA PCR Array as follows.**
   Note: For 384-well and 96-well array formats, a multichannel pipettor can be used to add reaction mix to the array. For the Rotor-Disc 100 format, a repeater pipettor or a QIAgility® can be used to load the array.

   For 384-well miScript miRNA PCR Array: add 10 µl per well.
   For 96-well miScript miRNA PCR Array: add 25 µl per well.
   For Rotor-Disc miScript miRNA PCR Array: add 20 µl per well.

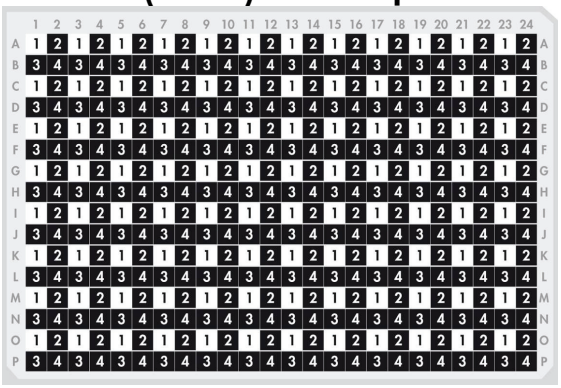
   Loading 384-well (4 x 96) Pathway-Focused miScript miRNA PCR Arrays:

   Note: Each Pathway-Focused miScript miRNA PCR Array contains 4 replicates of 96 assays that can be used for analysis of 4 samples. The

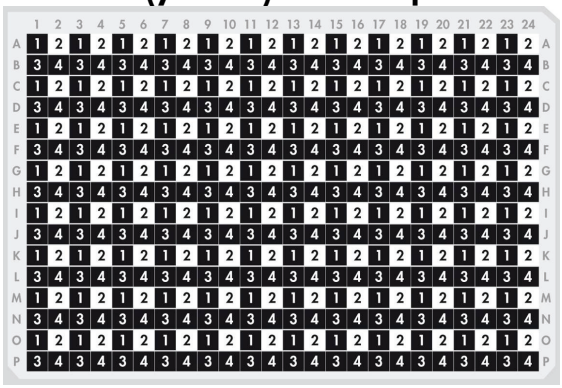
spacing between the tips of standard multichannel pipettors allows rows or columns to be skipped when adding each sample. Be sure to load each sample into the correct set of wells using a multichannel pipettor and the 384EZLoad Covers (provided). Use Figure 10 as a guide. Do not reuse 384EZLoad Covers.

- Place 384EZLoad Cover 1 (white) on the plate. Add 10 µl reaction mix for sample 1 to the open wells (odd number wells of rows A, C, E, G, I, K, M, and O). Remove and discard 384EZLoad Cover 1.
- Place 384EZLoad Cover 2 (yellow) on the plate. Add 10 µl reaction mix for sample 2 to the open wells (even number wells of rows A, C, E, G, I, K, M, and O). Remove and discard 384EZLoad Cover 2.
- Place 384EZLoad Cover 3 (black) on the plate. Add 10 µl reaction mix for sample 3 to the open wells (odd number wells of rows B, D, F, H, J, L, N, and P). Remove and discard 384EZLoad Cover 3.
- Place 384EZLoad Cover 4 (red) on the plate. Add 10 µl reaction mix for sample 4 to the open wells (even number wells of rows B, D, F, H, J, L, N, and P). Remove and discard 384EZLoad Cover 4.

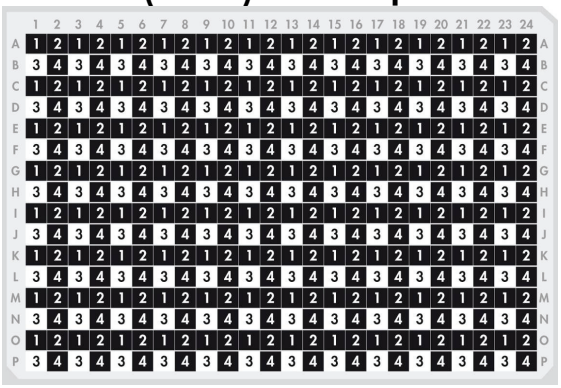
Cover 1 (white) for sample 1



Cover 2 (yellow) for sample 2



Cover 3 (black) for sample 3



Cover 4 (red) for sample 4

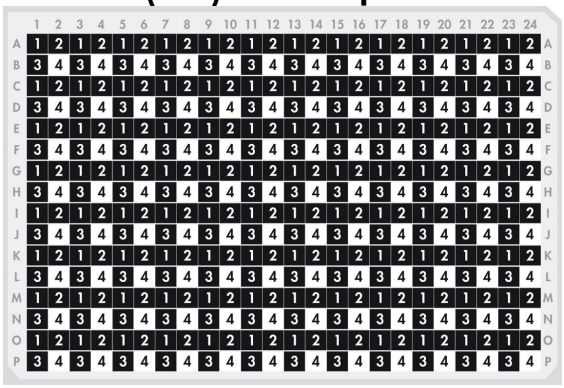


Figure 10. Loading Pathway-Focused miScript miRNA PCR Arrays, plate format E, G (384-well). Add 10 µl reaction mix for each of 4 samples into the staggered wells with the same number as indicated in the figure.

5. **Carefully, tightly seal the miScript miRNA PCR Array with Optical Thin-Wall 8-Cap Strips (Formats A and D), Optical Adhesive Film (Formats C, E, F, and G), or Rotor-Disc Heat-Sealing Film (Format R).**
6. **Centrifuge the PCR plate for 1 min at 1000 *g* at room temperature (15–25°C) to remove bubbles.**

   Note: This step is not necessary for reactions set up in Rotor-Discs.
7. **Program the real-time cycler according to Table 8.**

   Note: Perform dissociation curve analysis of the PCR product(s) to verify their specificity and identity. Dissociation curve analysis is an analysis step built into the software of real-time cyclers. Follow the instructions provided by the supplier.

Table 8. Cycling conditions for real-time PCR

Step	Time	Temperature	Additional comments
PCR Initial activation step	**15 min**	**95°C**	HotStarTaq DNA Polymerase is activated by this heating step.
3-step cycling:*†‡			
Denaturation	15 s	94°C	
Annealing	30 s	55°C	
Extension§	30 s	70°C	Perform fluorescence data collection.
Cycle number	40 cycles¶		Cycle number depends on the amount of template cDNA and abundance of the target.

* For Bio-Rad models CFX96 and CFX384: adjust the ramp rate to 1°C/s.

† For Eppendorf Mastercycler ep realplex models 2, 2S, 4, and 4S: for the Silver Thermoblock, adjust the ramp rate to 26%; for the Aluminum Thermoblock, adjust the ramp rate to 35%.

‡ If using a Roche LightCycler 480, adjust the ramp rate to 1°C/s.

§ Due to software requirements, the fluorescence detection step must be at least 30 s with the ABI PRISM® 7000 or 34 s with the Applied Biosystems 7300 and 7500.

¶ If using a Roche LightCycler 480, use 45 cycles.

8. **Place the plate/Rotor-Disc in the real-time cycler and start the cycling program.**
9. **Perform data analysis.**

Protocol: Data Analysis Using the $\Delta\Delta C_T$ Method of Relative Quantification for miScript miRNA PCR Arrays

This protocol describes the steps for analysis of data from miScript miRNA PCR Arrays. The first steps should be performed by the user. The later steps are performed by the free data analysis software. This data analysis software for miScript miRNA PCR Arrays is available at http://pcrdataanalysis.sabiosciences.com/mirna. Either the miScript miRNA PCR Array Web-based software or the miScript miRNA PCR Array Data Analysis Excel Template can be accessed. Both tools automatically perform quantification using the $\Delta\Delta C_T$ method of relative quantification and interpretation of the control wells. Results are presented in a tabular format, a scatter plot, a three-dimensional profile, and a volcano plot (when replicates are included).

Important point before starting

■ Text marked with a ■ denotes instructions for 96-well and 384-well plates (formats A, C, D, E, F, and G); text marked with a ▲ denotes instructions for 100-well Rotor-Discs (format R).

Procedure

Steps performed by the user

1. Define the baseline.

The baseline is the noise level in early cycles, where there is no detectable increase in fluorescence due to PCR products.

■ Use the "Linear View" of the amplification plot to determine the earliest visible amplification. Set the baseline from cycle 2 to 2 cycles before the earliest visible amplification. Do not use greater than cycle 15. The number of cycles used to calculate the baseline can be changed and should be reduced if high template amounts are used. For more information regarding real-time PCR data output, refer to Appendix A, page 48.

▲ For the Rotor-Gene Q, we recommend using the "Dynamic Tube" setting along with the "Slope Correct" and/or "Ignore First" settings. For more information, refer to the *Rotor-Gene Q User Manual*.

Note: Ensure that baseline settings are the same across all PCR runs associated with the same experiment to allow comparison of results.

2. Define the threshold.

The threshold should be set using a logarithmic amplification plot so that the log-linear range of the curve can be easily identified. Using the "Log View" of the amplification plot, place the threshold above the background signal but within the lower half of the log-linear range of the amplification

plot. The threshold should never be set in the plateau phase. The absolute position of the threshold is less critical than its consistent position across PCR runs.

■ Various PCR instruments (such as Applied Biosystems models 7500 and ViiA 7, and Stratagene models Mx3005P and Mx3000P) may require adjustment of the default "Manual C_T" threshold value of 0.2 to a lower value in order to analyze the data properly. Use a value of 0.02 as a starting point.

▲ For the Rotor-Gene Q, we recommend a C_T threshold value of approximately 0.02 in order to analyze the data properly.

Note: Ensure that threshold settings are the same across all PCR runs in the same analysis to allow comparison of results.

3. **Export C_T values according to the manual supplied with the real-time PCR instrument.**
4. **Access the free data analysis tools at http://pcrdataanalysis.sabiosciences.com/mirna.**

   Choose either the Web-based software or the Excel template and follow the instructions provided.

Steps performed by data analysis software

5. **All C_T values reported ■ as greater than 35 or as N/A (not detected) are changed to 35 or ▲ as greater than 33 or as N/A (not detected) are changed to 33.**

   At this point, any C_T value equal to ■ 35 or ▲ 33 is considered a negative call.

6. **C_T values of the positive PCR control wells (PPC) are examined.**

   If the RNA sample is of high quality, the cycling program has been correctly run, and the thresholds have been correctly defined, the value of C_T^{PPC} should be ■ 19 ± 2 or ▲ 15 ± 2 across all arrays or samples.

7. **C_T values of the reverse transcription control (miRTC) are examined using the values for the positive PCR control (PPC) by calculating ΔC_T = AVG C_T^{miRTC} – AVG C_T^{PPC}.**

   If this value is less than 7, then no inhibition of the reverse-transcription reaction is apparent. No action is needed. If this value is greater than 7, there is evidence of impurities that may have inhibited the reverse transcription reaction. See the "Troubleshooting Guide", page 45.

   Note: The ΔC_T = AVG C_T^{miRTC} – AVG C_T^{PPC} calculation is specific for Pathway-Focused miScript miRNA PCR Arrays. For miRNome miScript miRNA PCR Arrays, as the cDNA is divided among significantly more wells, a correction factor is introduced into the calculation according to the

number of plates/Rotor-Discs that are being used. This corrects for the dilution of the miRTC. The calculation is performed as shown in Table 9.

Table 9. $\Delta C_T^{(miRTC\text{-}PPC)}$ calculation for miRNome miScript miRNA PCR Arrays

Number of plates/Rotor-Discs				
384-well	**96-well**	**Rotor-Disc 100**	**Correction factor**	**$\Delta C_T^{(miRTC\text{-}PPC)}$ calculation**
1	4	4	1.1	(AVG C_T^{miRTC} – 1.1) – (AVG C_T^{PPC})
2	8	8	2.1	(AVG C_T^{miRTC} – 2.1) – (AVG C_T^{PPC})
3	12	12	2.7	(AVG C_T^{miRTC} – 2.7) – (AVG C_T^{PPC})
4	16	16	3.1	(AVG C_T^{miRTC} – 3.1) – (AVG C_T^{PPC})

8. **ΔC_T value for each mature miRNA profiled in the plate is calculated using the formula $\Delta C_T = C_T^{miRNA} – AVG\ C_T^{SN1/2/3/4/5/6}$.**

   Note: Choose an appropriate snoRNA/snRNA control for normalization. Six snoRNA/snRNA controls (SN1–6) are included on each array. Make sure that the selected controls are not influenced by the experimental conditions. If one or more snoRNA/snRNA have been previously independently identified and if the miScript miRNA PCR Array reproduces those results, use the average of their C_T values in the equation above. If an appropriate snoRNA/snRNA has not been previously identified, use the average C_T value of all the snoRNA/snRNA. When biological and/or technical replicates are performed, calculate the average ΔC_T value of each snoRNA/snRNA (each well) across those replicate arrays for each treatment group.

9. **$\Delta\Delta C_T$ for each miRNA across 2 miScript miRNA PCR Arrays or 2 samples is calculated using the formula: $\Delta\Delta C_T = \Delta C_T$ (sample 2) – ΔC_T (sample 1) where sample 1 is the control sample and sample 2 is the experimental sample.**

10. **Fold-change for each gene from sample 1 to sample 2 is calculated as $2^{(-\Delta\Delta CT)}$.**

    Optional: If the fold-change is greater than 1, the result may be reported as a fold upregulation. If the fold-change is less than 1, the negative inverse of the result may be reported as a fold downregulation.

11. **Fold-changes are presented by the data analysis tool in a variety of formats, including a tabular format, a scatter plot, a three-dimensional profile, and a volcano plot (when replicates are included).**

Protocol: cDNA Quality Control Prior to Profiling Mature miRNA

Use of the miScript miRNA QC PCR Array enables testing of the quality of cDNA prepared using miScript HiSpec Buffer, saving time and reagents. Only 5 µl diluted cDNA sample is required for quality control using the miScript miRNA QC PCR Array. cDNA prepared using the miScript II RT Kit with miScript HiSpec Buffer is the appropriate starting material for this protocol. This protocol describes quality control of multiple cDNA samples using the miScript SYBR Green PCR Kit and miScript miRNA QC PCR Array prior to miRNA profiling using miScript miRNA PCR Arrays. In total, 32 cDNA samples can be analyzed on one 384-well miScript miRNA QC PCR Array, 8 cDNA samples can be analyzed on one 96-well miScript miRNA QC PCR Array, and 8 cDNA samples can be analyzed on one Rotor-Disc 100 miScript miRNA QC PCR Array.

Important points before starting

- The PCR must start with an **initial incubation step of 15 minutes at 95°C** to activate HotStarTaq DNA Polymerase (included in 2x QuantiTect SYBR Green PCR Master Mix).
- Only cDNA template prepared using miScript HiSpec Buffer should be used with this protocol.
- Ensure cDNA samples were prepared according to the protocol on page 26, and that the 20 µl cDNA synthesis reaction has been diluted appropriately (see Table 5, page 29).
- Do not vortex template cDNA or any of the components of the miScript SYBR Green PCR Kit.
- The standard miScript miRNA PCR Array reaction volumes are 10 µl per well for a 384-well plate, 25 µl per well for a 96-well plate, and 20 µl per well for a 100-well Rotor-Disc.
- If using the iCycler iQ, iQ5, or MyiQ, well factors must be collected at the beginning of each experiment. Well factors are used to compensate for any system or pipetting nonuniformity. For details, refer to the user manual supplied with the instrument or Technical Information: Using QuantiTect SYBR Green Kits on Bio-Rad cyclers available at www.qiagen.com.

Procedure

1. **Thaw 2x QuantiTect SYBR Green PCR Master Mix, 10x miScript Universal Primer, template cDNA, and RNase-free water at room temperature (15–25°C). Mix the individual solutions.**

2. **Prepare a reaction mix according to Table 10. Mix gently and thoroughly.**

   Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the real-time cycler.

Table 10. Reaction mix for miScript miRNA QC PCR Arrays*

Array format: Component	384-well Formats E, G†	96-well Formats A, C, D, F†	Rotor-Disc 100 Format R†
2x QuantiTect SYBR Green PCR Master Mix‡	75 µl	175 µl	150 µl
10x miScript Universal Primer	15 µl	35 µl	30 µl
RNase-free water	55 µl	135 µl	115 µl
Template cDNA	5 µl	5 µl	5 µl
Total volume	**150 µl**	**350 µl**	**300 µl**

* These volumes provide a 10 µl per well reaction volume for a 384-well plate, 25 µl per well reaction volume for a 96-well plate, and 20 µl per well reaction volume for a 100-well Rotor-Disc.

† Volumes shown are sufficient for one cDNA template. In total, 8 cDNA samples can be analyzed on one 96-well miScript miRNA QC PCR Array, 8 cDNA samples can be analyzed on one Rotor-Disc 100 miScript miRNA QC PCR Array, and 32 cDNA samples can be analyzed on one 384-well miScript miRNA QC PCR Array.

‡ No optimization of the Mg^{2+} concentration is required. The final Mg^{2+} concentration provided by 2x QuantiTect SYBR Green PCR Master Mix gives optimal results.

3. **Carefully remove the miScript miRNA QC PCR Array from its sealed bag.**
4. **Add reaction mix to the wells of the miScript miRNA QC PCR Array as follows.**

   For 384-well miScript miRNA PCR Array: add 10 µl per well.
   For 96-well miScript miRNA PCR Array: add 25 µl per well.
   For Rotor-Disc 100 miScript miRNA PCR Array: add 20 µl per well.
5. **Carefully, tightly seal the miScript miRNA PCR Array with Optical Thin-Wall 8-Cap Strips (Formats A and D), Optical Adhesive Film (Formats C, E, F, and G), or Rotor-Disc Heat-Sealing Film (Format R).**
6. **Centrifuge the PCR plate for 1 min at 1000 *g* at room temperature (15–25°C) to remove bubbles.**

   Note: This step is not necessary for reactions set up in Rotor-Discs.

7. **Program the real-time cycler according to Table 11.**

   Note: Perform dissociation curve analysis of the PCR product(s) to verify their specificity and identity. Dissociation curve analysis is an analysis step built into the software of real-time cyclers. Follow the instructions provided by the supplier.

Table 11. Cycling conditions for real-time PCR

Step	Time	Temperature	Additional comments
PCR Initial activation step	**15 min**	**95°C**	HotStarTaq DNA Polymerase is activated by this heating step.
3-step cycling:*†‡			
Denaturation	15 s	94°C	
Annealing	30 s	55°C	
Extension§	30 s	70°C	Perform fluorescence data collection.
Cycle number	40 cycles¶		Cycle number depends on the amount of template cDNA and abundance of the target.

* For Bio-Rad models CFX96 and CFX384: adjust the ramp rate to 1°C/s.

† For Eppendorf Mastercycler ep realplex models 2, 2S, 4, and 4S: for the Silver Thermoblock, adjust the ramp rate to 26%; for the Aluminum Thermobock, adjust the ramp rate to 35%.

‡ If using a Roche LightCycler 480, adjust the ramp rate to 1°C/s.

§ Due to software requirements, the fluorescence detection step must be at least 30 s with the ABI PRISM 7000 or 34 s with the Applied Biosystems 7300 and 7500.

¶ If using a Roche LightCycler 480, use 45 cycles.

8. **Place the plate/Rotor-Disc in the real-time cycler and start the cycling program.**
9. **Perform data analysis.**

Protocol: Data Analysis for Quality Control Using miScript miRNA QC PCR Arrays

This protocol describes the steps for analysis of data from miScript miRNA QC PCR Arrays. The first steps should be performed by the user. The later steps are performed by the free data analysis software. Free data analysis software for miScript miRNA QC PCR Arrays is available at http://pcrdataanalysis.sabiosciences.com/mirna. Either the miScript miRNA PCR Array Web-based software or the miScript miRNA PCR Array Data Analysis Excel Template can be accessed. Both tools automatically perform quantification using the $\Delta\Delta C_T$ method of relative quantification and interpretation of the control wells.

Important point before starting

■ Text marked with a ■ denotes instructions for 96-well and 384-well plates (formats A, C, D, E, F, and G); text marked with a ▲ denotes instructions for 100-well Rotor-Discs (format R).

Procedure

Steps performed by the user

1. Define the baseline.

The baseline is the noise level in early cycles, where there is no detectable increase in fluorescence due to PCR products.

■ Use the "Linear View" of the amplification plot to determine the earliest visible amplification. Set the baseline from cycle 2 to 2 cycles before the earliest visible amplification. Do not use greater than cycle 15. The number of cycles used to calculate the baseline can be changed and should be reduced if high template amounts are used. For more information regarding real-time PCR data output, refer to Appendix A, page 48.

▲ For the Rotor-Gene Q, we recommend using the "Dynamic Tube" setting along with the "Slope Correct" and/or "Ignore First" settings. For more information, refer to the *Rotor-Gene Q User Manual*.

Note: Ensure that baseline settings are the same across all PCR runs in the same analysis to allow comparison of results.

2. Define the threshold.

The threshold should be set using a logarithmic amplification plot so that the log-linear range of the curve can be easily identified. Using the "Log View" of the amplification plot, place the threshold above the background signal but within the lower half of the log-linear range of the amplification plot. The threshold should never be set in the plateau phase. The absolute

position of the threshold is less critical than its consistent position across PCR runs.

■ Various PCR instruments (such as Applied Biosystems models 7500 and ViiA 7, and Stratagene models Mx3005P and Mx3000P) may require adjustment of the default "Manual C_T" threshold value of 0.2 to a lower value in order to analyze the data properly. Use a value of 0.02 as a starting point.

▲ For the Rotor-Gene Q, we recommend a C_T threshold value of approximately 0.02 in order to analyze the data properly.

Note: Ensure that threshold settings are the same across all PCR runs in the same analysis to allow comparison of results.

3. **Export C_T values according to the manual supplied with the real-time PCR instrument.**
4. **Access the free data analysis tools at http://pcrdataanalysis.sabiosciences.com/mirna.**

   Choose either the Web-based software or the Excel template and follow the instructions provided.

Steps performed by data analysis software

5. **All C_T values reported ■ as greater than 35 or as N/A (not detected) are changed to 35 or ▲ as greater than 33 or as N/A (not detected) are changed to 33.**

   At this point, any C_T value equal to ■ 35 or ▲ 33 is considered a negative call.

6. **C_T values of the positive PCR control wells (PPC) are examined.**

   If the RNA sample is of high quality, the cycling program has been correctly run, and the thresholds have been correctly defined, the value of C_T^{PPC} should be ■ 19 ± 2 or ▲ 15 ± 2 across all arrays or samples.

7. **C_T values of the reverse transcription control (miRTC) are examined using the values for the positive PCR control (PPC) by calculating ΔC_T = AVG C_T^{miRTC} – AVG C_T^{PPC} – correction factor.**

   If this value is less than 7, then no inhibition of the reverse-transcription reaction is apparent. No action is needed. If this value is greater than 7, there is evidence of impurities that may have inhibited the reverse transcription reaction. See the "Troubleshooting Guide", page 45.

   Note: As the cDNA will be divided among different numbers of wells in the downstream experiment, a correction factor is introduced into the calculation according to the number of plates/Rotor-Discs that will used. This corrects for the dilution of the miRTC. The calculation is performed as shown in Table 12.

Table 12. $\Delta C_T^{(miRTC\ -PPC)}$ calculations for miScript miRNA QC PCR Arrays

Intended use of cDNA	Correction factor	$\Delta C_T^{(miRTC\ -PPC)}$ calculation
Pathway-Focused miScript miRNA PCR Array	1.5	(AVG C_T^{miRTC} – 1.5) – (AVG C_T^{PPC})
Whole miRNome: 1 x 384-well plate or 4 x 96-well plates/Rotor-Discs	0.5	(AVG C_T^{miRTC} – 0.5) – (AVG C_T^{PPC})
Whole miRNome: 2 x 384-well plate or 8 x 96-well plates/Rotor-Discs	1.5	(AVG C_T^{miRTC} – 1.5) – (AVG C_T^{PPC})
Whole miRNome: 3 x 384-well plate or 12 x 96-well plates/Rotor-Discs	2.0	(AVG C_T^{miRTC} – 2.0) – (AVG C_T^{PPC})
Whole miRNome: 4 x 384-well plate or 16 x 96-well plates/Rotor-Discs	2.5	(AVG C_T^{miRTC} – 2.5) – (AVG C_T^{PPC})

8. C_T values of the snoRNA/snRNA controls (SN1–6) are examined.

Note: These small RNAs have been verified to have relatively stable expression levels across tissues and cell types. Nevertheless, snoRNA/snRNA control C_T values remain sample dependent and should be checked to determine whether their expression is consistent across the samples that are being analyzed. If the expression of a particular control is not consistent across experimental samples, that control should not be used for data normalization. For examples of C_T values associated with various tissue types, refer to the miScript PCR Controls application data at www.qiagen.com/miRNAControls.

9. *C. elegans* miR-39 miScript Primer Assay C_T values (Ce) are examined.

The *C. elegans* miR-39 miScript Primer Assay should only result in C_T values above the threshold if Syn-cel-miR-39 miScript miRNA Mimic has been spiked in to the sample prior to RNA purification. For more information, see the *QIAGEN Supplementary Protocol: Purification of total RNA, including*

small RNAs, from serum or plasma using the miRNeasy Mini Kit available at www.qiagen.com/miRNeasyMiniResources.

10. **If all criteria described above are met, cDNA samples are of sufficient quality for analysis. Proceed with miScript miRNA PCR Array experiments.**

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
Evidence of poor reverse transcription efficiency (value of AVG C_T^{miRTC} – AVG C_T^{PPC} > 7)	
a) Poor quality RNA	Check the A_{260}:A_{280} and A_{260}:A_{230} ratios of the RNA samples. Be sure to perform the dilutions for spectrophotometry in RNase-free 10 mM Tris·Cl, pH 7.5. If necessary, repurify RNA with a spin-column based clean up method, such as the miRNeasy Mini Kit (cat. no. 217004).
b) Calculation did not include correction factor	If using miRNome miScript miRNA PCR Arrays or miScript miRNA QC PCR Arrays, be sure to include the correction factors detailed in Table 9, page 37 or Table 12, page 43.
Evidence of poor, overall PCR amplification efficiency (AVG C_T^{PPC} varies by more than 2 across arrays and/or is greater than 21 for 96-well and 384-well plates or 17 for 100-well Rotor-Discs)	
a) Variation in instrument sensitivity	Different instruments have different levels of sensitivity. If an average C_T^{PPC} value of 19 ± 2 for 96-well and 384-well plates or 15 ± 2 for 100-well Rotor-Discs is difficult to obtain for the instrument used, the observed average C_T^{PPC} value should be acceptable as long as it does not vary by more than 2 cycles between arrays being compared.
b) HotStarTaq DNA Polymerase not activated with a hot start	Be sure that the initial heat activation step at 95°C took place for 15 minutes, and that all other cycle parameters were performed according to the protocol.

	Comments and suggestions
c) Poor quality RNA that may contain PCR inhibitors	Check the A_{260}:A_{280} and A_{260}:A_{230} ratios of the RNA samples. Be sure to perform the dilutions for spectrophotometry in RNase-free 10 mM Tris·Cl, pH 7.5. If necessary, repurify RNA with a spin-column based clean up method, such as the miRNeasy Mini Kit (cat. no. 217004).

No product, or product detected late in real-time PCR (indicative of problems occurring during reverse transcription)

a) Pipetting error or missing reagent when setting up reverse-transcription reaction	Check the pipets used for experimental setup. Mix all reagents well after thawing and repeat the reverse-transcription reaction.
b) Incorrect setup of reverse-transcription reaction	Be sure to set up the reaction on ice.
c) Poor quality or incorrect amount of template RNA for reverse-transcription reaction	Check the concentration, integrity, and purity of the template RNA before starting the protocol. Mix well after thawing the template RNA. Even minute amounts of RNases can affect synthesis of cDNA and sensitivity in RT-PCR, particularly with small amounts of RNA.
d) RNA concentration too high or too low	See Table 3, page 27 for recommended RNA amounts.
e) RNA denatured	Denaturation of the template RNA is not necessary. If denaturation was performed, the integrity of the RNA may be affected.
f) Incubation temperature too high	Reverse transcription should be carried out at 37°C. Higher temperatures may reduce the length of cDNA products or the activity of miScript Reverse Transcriptase Mix. Check the temperature of your heating block or water bath.

	Comments and suggestions
No product, or product detected late in real-time PCR, or only primer–dimers detected (indicative of problems occurring during real-time PCR)	
a) PCR annealing time too short	Use the annealing time specified in the protocol.
b) PCR extension time too short	Use the extension time specified in the protocol.
c) Pipetting error or missing reagent when setting up PCR	Check the concentrations and storage conditions of reagents, including primers and cDNA.
d) HotStarTaq DNA Polymerase not activated with a hot start	Ensure that the cycling program includes the hot start activation step for HotStarTaq DNA polymerase; for details, check the protocol.
e) No detection activated	Check that fluorescence detection was activated in the cycling program.
f) Wrong detection step	Ensure that fluorescence detection takes place during the extension step of the PCR cycling program.
g) Wrong dye layer/filter chosen	Ensure that the appropriate layer/filter is activated.
h) Insufficient starting template	Increase the amount of template cDNA.
No linearity in ratio of C_T value/crossing point to log of the template amount	
a) Template amount too high	Do not exceed maximum recommended amounts of template cDNA. For details, see the protocol.
b) Template amount too low	Increase amount of template RNA.
Varying fluorescence intensity	
a) Real-time cycler contaminated	Decontaminate the real-time cycler according to the supplier's instructions.
b) Real-time cycler no longer calibrated	Recalibrate the real-time cycler according to the supplier's instructions.

Appendix A: Real-Time PCR Data Output and Dissociation Curve Analysis

In a typical amplification plot resulting from a real-time PCR reaction, fluorescence is plotted against the number of cycles, producing sigmoidal-shaped plots (when using a linear scale). The threshold cycle (C_T) serves as a tool for calculation of the starting template amount in each sample. This is the cycle in which there is the first detectable increase in fluorescence. There may be variation in how determination of C_T values is carried out depending on the real-time PCR cycler that is used.

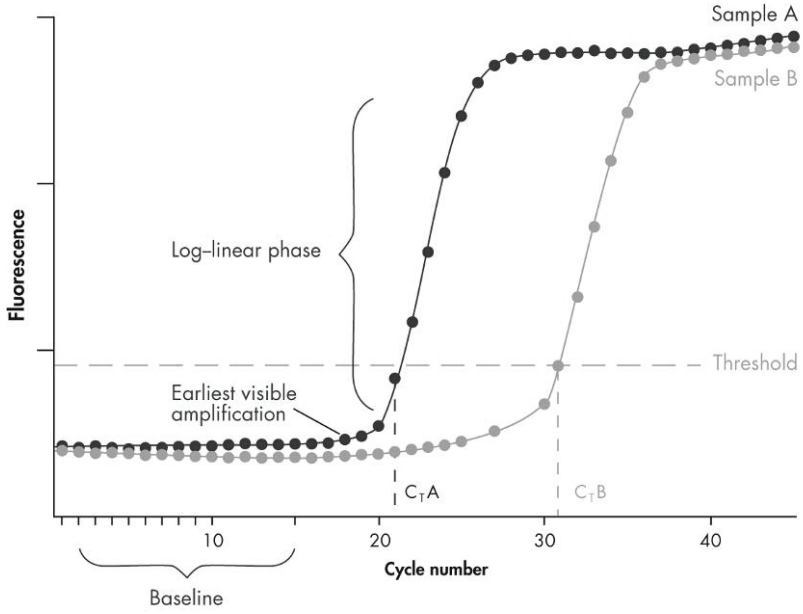


Figure 11. Amplification plot. Amplification plots showing increases in fluorescence from 2 samples (A and B). Sample A contains a higher amount of starting template than sample B.

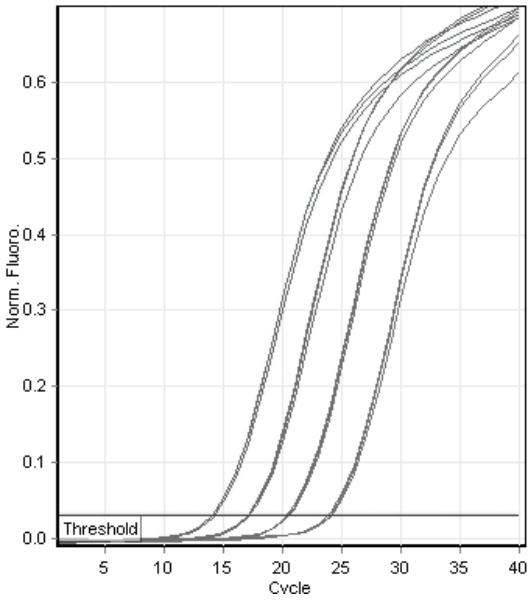


Figure 12. Typical amplification plot. Amplification plot after quantification of a range of amounts of miR-21. Real-time PCR was performed using the Rotor-Gene Q.

Dissociation curve analysis

A dissociation curve analysis of PCR product(s) may be optionally performed to aid in verifying their specificity and identity. Dissociation curve analysis is an analysis step built into the software of real-time cyclers. Follow instructions provided by the supplier.

To carry out dissociation curve analysis, the temperature is increased very slowly from a low temperature (e.g., 65°C) to a high temperature (e.g., 95°C). At low temperatures, PCR products are double stranded, so SYBR Green I dye binds to them and fluorescence is high. However at high temperatures, PCR products are denatured, resulting in rapid decreases in fluorescence.

The fluorescence is measured continuously as the temperature is increased and the fluorescence values are plotted against temperature. A curve is produced, because fluorescence decreases slightly through the lower end of the temperature range, but decreases much more rapidly at higher temperatures as the dissociation temperatures of nonspecific and specific PCR products are reached. The detection systems calculate the first derivatives of the curves, resulting in curves with peaks at the respective T_ms (Figures 13 and 14). Curves with peaks at a T_m lower than that of the specific PCR product indicate the formation of primer–dimers, while diverse peaks with different T_ms or plateaus indicate production of nonspecific products or a smear.

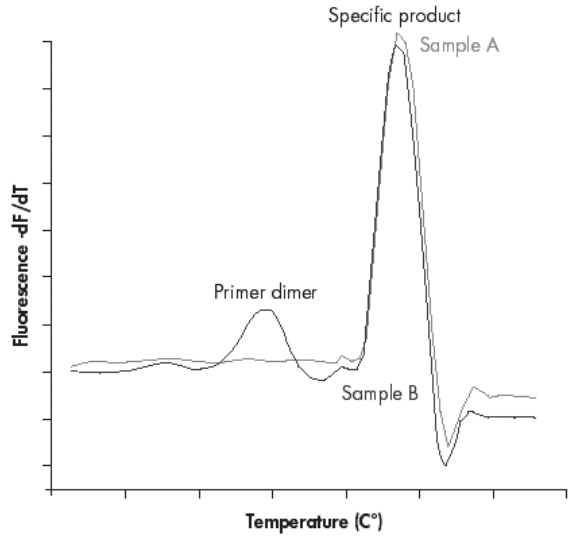


Figure 13. Dissociation curve analysis. Dissociation curve analysis of 2 samples (A and B). Sample A yields only 1 peak, resulting from the specific amplification product (primer–dimers not coamplified). Sample B shows a peak from the specific product and a peak at a lower temperature from amplification of primer–dimers.

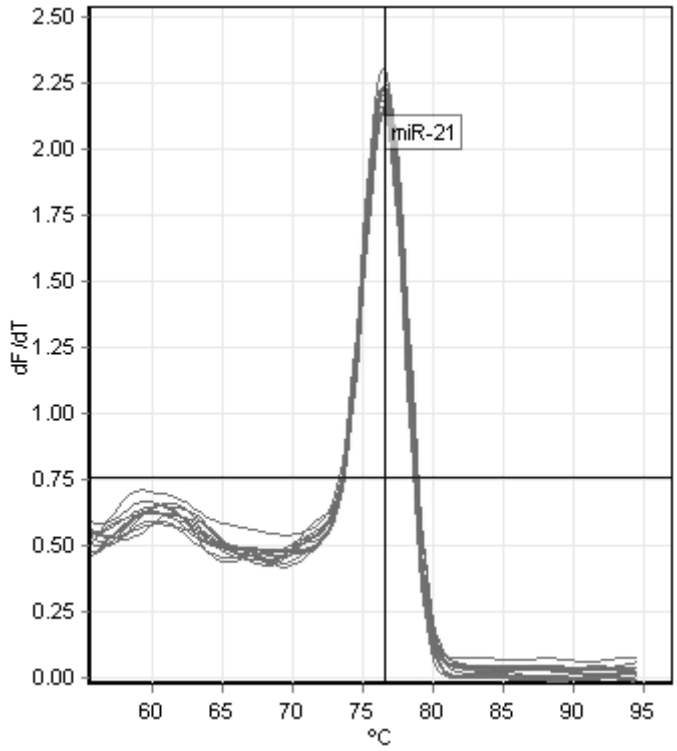


Figure 14. miRNA dissociation curve. Dissociation curve analysis of an miR-21 PCR product showing a single peak from the specific amplification product. Dissociation curve analysis was performed using the Rotor-Gene Q.

Appendix B: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to degrade RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

To remove RNase contamination from bench surfaces, nondisposable plasticware, and laboratory equipment (e.g., pipets and electrophoresis tanks), use of RNaseKiller (cat. no. 2500080) from 5 PRIME (www.5prime.com) is recommended. RNase contamination can alternatively be removed using general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 52), or rinse with chloroform* if the plasticware is chloroform-resistant. To decontaminate electrophoresis tanks, clean with detergent (e.g., 0.5% SDS),* rinse with RNase-free water, rinse with ethanol (if the tanks are ethanol-resistant), and allow to dry.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material data sheets (MSDSs), available from the product supplier.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed, and oven baked at 240°C for 4 hours or more (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate), as described in "Solutions" below.

Solutions

Note: QIAGEN solutions, such as miScript Nucleics Mix, miScript HiFlex Buffer, miScript HiSpec Buffer, and RNase-free water, are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material data sheets (MSDSs), available from the product supplier.

Appendix C: Preparation, Quantification, and Storage of RNA

RNA preparation and quality

Since PCR consists of multiple rounds of enzymatic reactions, it is more sensitive to impurities such as proteins, phenol/chloroform, salts, and EDTA than single-step enzyme-catalyzed reactions. Purity of nucleic acid templates is particularly important for real-time PCR, since contaminants can interfere with fluorescence detection. See Table 2, page 24 for kits recommended for the purification of total RNA that includes miRNA. For more information about kits for miRNA purification, visit www.qiagen.com/miRNA.

Determining concentration and purity of RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. The sample should be diluted in water since the relationship between absorbance and concentration (A_{260} reading of 1 = 40 µg/ml RNA) is based on an extinction coefficient calculated for RNA in water. To ensure significance, readings should fall between 0.15 and 1.0.

Note that absorbance measurements cannot discriminate between DNA and RNA. Depending on the method used for RNA preparation, RNA may be contaminated with DNA, and this will result in misleadingly high A_{260} values.

The ratios between the absorbance values at 260 nm and 280 nm and at 260 nm and 230 nm give an estimate of RNA purity. To determine RNA purity, we recommend measuring absorbance in 10 mM Tris·Cl, pH 7.5.* Pure RNA has an A_{260}/A_{280} ratio of 1.9–2.1 and an A_{260}/A_{230} ratio of 2.0–2.2.† Lower ratios indicate the presence of contaminants such as proteins.

Storage of RNA

Purified RNA should be stored at –20°C or –70°C in RNase-free water. When purified using QIAGEN systems, no degradation is detectable for at least 1 year under these conditions. Diluted solutions of RNA (e.g., dilution series used as standards) should be stored in aliquots and thawed once only. We recommend storage of aliquots in siliconized tubes where possible. This avoids adsorption of the RNA to the tube walls, which would reduce the concentration of RNA in solution.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

† A_{260}:A_{280} values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris·Cl, pH 7.5) with some spectrophotometers.

DNA contamination in RNA samples purified from different tissues

Depending on the type of tissue used as starting material for RNA purification, a fluorescent signal may also be generated in "no RT" control reactions. When RNA is purified from tissues that contain large amounts of DNA, such as spleen or thymus, the level of DNase treatment required may be higher than for other tissues. For such tissues, we recommend performing a DNase digestion (using the QIAGEN RNase-Free DNase Set, cat. no. 79254) when using the miRNeasy Mini and miRNeasy 96 Kits for RNA purification.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
miScript II RT Kit (12)	For 12 cDNA synthesis reactions: miScript Reverse Transcriptase Mix, 10x miScript Nucleics Mix, 5x miScript HiSpec Buffer, 5x miScript HiFlex Buffer, RNase-Free Water	218160
miScript II RT Kit (50)	For 50 cDNA synthesis reactions: miScript Reverse Transcriptase Mix, 10x miScript Nucleics Mix, 5x miScript HiSpec Buffer, 5x miScript HiFlex Buffer, RNase-Free Water	218161
miScript SYBR Green PCR Kit (200)	For 200 reactions: QuantiTect SYBR Green PCR Master Mix, miScript Universal Primer	218073
miScript SYBR Green PCR Kit (1000)	For 1000 reactions: QuantiTect SYBR Green PCR Master Mix, miScript Universal Primer	218075
miScript Primer Assay (100)	10x miScript Primer Assay (contains one miRNA-specific primer)	Varies*
Pathway-Focused miScript miRNA PCR Array	Array of assays for a pathway, disease, or gene family for human, mouse, rat, or dog miRNAs; available in 96-well, 384-well, or Rotor-Disc 100 format	Varies
miRNome miScript miRNA PCR Array	Array of assays for the complete human, mouse, rat, or dog miRNome; available in 96-well, 384-well, or Rotor-Disc 100 format	Varies
miScript miRNA QC PCR Array	Array of quality control assays for human, mouse, rat, or dog miRNAs; available in 96-well, 384-well, or Rotor-Disc 100 format	Varies
RT^2 PCR Array Loading Reservoir	12 x 5 ml capacity, irradiation-sterilized loading reservoirs	338162

*Visit www.qiagen.com/GeneGlobe to search for and order these products.

Product	Contents	Cat. no.
384EZLoad Covers	Pack of 4 color-coded covers for loading 384-well plates	338125
Related products		
miRNeasy Mini Kit (50)	For 50 preps: 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	217004
miRNeasy 96 Kit (4)	For 4 x 96 preps: 4 RNeasy 96 plates, Collection Microtubes (racked), Elution Microtubes CL, Caps, S-Blocks, AirPore Tape Sheets, QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	217061
miRNeasy FFPE Kit (50)	50 RNeasy MinElute® Spin Columns, Collection Tubes, Proteinase K, RNase-Free DNase I, DNase Booster Buffer, RNase-Free Buffers, RNase-Free Water	217504
PAXgene Tissue miRNA Kit (50)	For 50 RNA preps: PAXgene RNA MinElute Spin Columns, PAXgene Shredder Spin Columns, Processing Tubes, Microcentrifuge Tubes, Carrier RNA, RNase-Free DNase, and RNase-Free Buffers; to be used with PAXgene Tissue Containers	766134
PAXgene Tissue Containers (10)	For collection, fixation, and stabilization of 10 samples: 10 Prefilled Reagent Containers, containing PAXgene Tissue Fix and PAXgene Tissue Stabilizer	765112
PAXgene Blood miRNA Kit (50)	For 50 RNA preps: PAXgene Spin Columns, PAXgene Shredder Spin Columns, Processing Tubes, Microcentrifuge Tubes, RNase-Free DNase, RNase-Free Reagents and Buffers; to be used with PAXgene Blood RNA Tubes (available from BD, cat. no. 762165)	763134

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Notes

www.qiagen.com

Australia ■ Orders 1-800-243-800 ■ Fax 03-9840-9888 ■ Technical 1-800-243-066

Austria ■ Orders 0800-28-10-10 ■ Fax 0800-28-10-19 ■ Technical 0800-28-10-11

Belgium ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

Brazil ■ Orders 0800-557779 ■ Fax 55-11-5079-4001 ■ Technical 0800-557779

Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ Orders 86-21-3865-3865 ■ Fax 86-21-3865-3965 ■ Technical 800-988-0325

Denmark ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

Finland ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

France ■ Orders 01-60-920-926 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930 ■ Offers 01-60-920-928

Germany ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Hong Kong ■ Orders 800 933 965 ■ Fax 800 930 439 ■ Technical 800 930 425

Ireland ■ Orders 1800 555 049 ■ Fax 1800 555 048 ■ Technical 1800 555 061

Italy ■ Orders 800-789-544 ■ Fax 02-334304-826 ■ Technical 800-787980

Japan ■ Telephone 03-6890-7300 ■ Fax 03-5547-0818 ■ Technical 03-6890-7300

Korea (South) ■ Orders 080-000-7146 ■ Fax 02-2626-5703 ■ Technical 080-000-7145

Luxembourg ■ Orders 8002-2076 ■ Fax 8002-2073 ■ Technical 8002-2067

Mexico ■ Orders 01-800-7742-639 ■ Fax 01-800-1122-330 ■ Technical 01-800-7742-436

The Netherlands ■ Orders 0800-0229592 ■ Fax 0800-0229593 ■ Technical 0800-0229602

Norway ■ Orders 800-18859 ■ Fax 800-18817 ■ Technical 800-18712

Singapore ■ Orders 1800-742-4362 ■ Fax 65-6854-8184 ■ Technical 1800-742-4368

Spain ■ Orders 91-630-7050 ■ Fax 91-630-5145 ■ Technical 91-630-7050

Sweden ■ Orders 020-790282 ■ Fax 020-790582 ■ Technical 020-798328

Switzerland ■ Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

UK ■ Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

USA ■ Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)

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